Alberta Environment Cyanotoxin Program

Status Report

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Alberta Environment

Cyanotoxin Program

Status Report

Prepared by:

Ron Zurawell, Ph.D., P.Biol.
Limnologist/Water Quality Specialist

Water Policy Branch

Environmental Assurance Division
Alberta Environment

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Any comments, questions, or suggestions regarding the content of this document may be directed to:

Water Policy Branch
Alberta Environment
7th Floor, Oxbridge Place
9820 – 106th Street
Edmonton, Alberta T5K 2J6
Phone: (780) 427-6278
Fax: (780) 422-6712

Additional copies of this document may be obtained by contacting:

Information Centre
Alberta Environment
Main Floor, Oxbridge Place
9820 – 106th Street
Edmonton, Alberta T5K 2J6
Phone: (780) 427-2700
Fax: (780) 422-4086
Email: env.infocent@gov.ab.ca

EXECUTIVE SUMMARY

Alberta's nutrient-rich lakes and reservoirs often experience blooms of cyanobacteria (a.k.a. blue-green algae) during summer and early fall. Common species of cyanobacteria can produce potent liver and/or nerve toxins. Microcystins (MCYSTs) are thought to be the most common toxins produced by cyanobacteria and exert toxicity by severely damaging liver structure and function. They have been linked to high incidence of primary liver (hepatocellular carcinoma) and colorectal cancers in some countries.

Cyanotoxin monitoring was incorporated into Alberta's Integrated Lake and Reservoir Monitoring Program in 2005 with the goal of determining the prevalence of MCYST in Alberta. In addition, we investigate the occurrence of multiple MCYST analogues, a potent neurotoxin anatoxin-a (ATX-a), and a potentially toxic amino acid, β-*N*-methylamino-L-alanine (BMAA) recently reported to be produced by cyanobacteria.

Monitoring data collected during 4 open water seasons (2005-2008), reveal that MCYSTs are prevalent in a majority of Alberta's lakes and reservoirs. Though appearing more common and at higher concentrations in nutrient-rich, eutrophic and hypereutrophic waterbodies, MCYSTs did occur periodically in lower nutrient, oligo- and mesotrophic waters. Metalimnetic blooms of specific cyanobacteria may account for toxin in these low nutrient environments.

Average off-shore (open-water) concentrations can be elevated, occasionally exceeding the draft recreational water quality (RWQ) guideline of 20 μg/L. Though not determined in this study, concentrations in near-shore bloom accumulations do become much (2 orders of magnitude) higher than that in open-water areas.

MCYST concentration is correlated with the abundance of toxin-producing cyanobacteria. Cyanobacterial growth and reproduction and the onset, duration, and severity of surface blooms are likely influenced by climate-mediated water column warming and stability. Warmer years could have greater occurrence and concentrations of microcystins.

In a subset of samples, multiple MCYST analogues were detected. While microcystin-LR (MCLR) appears to be most common, other toxin congeners occurred at higher concentrations and in the absence of MCLR. Toxicity of MCYST analogues can vary greatly with specific chemical structure. These findings challenge the applicability or suitability of the current Canadian Drinking Water Quality Guideline specifying only MCLR and not total MCYST concentration.

In contrast to MCYST, ATX-a occurred infrequently and at low concentrations in Alberta's lakes and reservoirs monitored in 2005 and was not detected during the summer of 2006.

Analytical methods for determining BMAA in surface water samples have been developed. Initial work indicates this compound may occur in Alberta eutrophic surface waters. Studies on the prevalence of BMAA are ongoing.

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GLOSSARY OF TERMS AND ACRONYMS

µg MCLR eq./L	Unit of measure estimating micrograms of total microcystin concentration per liter water based on a toxicity response equivalent to a known concentration of microcystin-LR.
ACFT	The Alberta Centre for Toxicology, University of Calgary.
AENV	Alberta Environment
ALMS	The Alberta Lake Management Society
ALS/PDC	Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex: a neurodegenerative disorder with symptoms consistent with or similar to ALS and Parkinson's diseases.
API	Alberta Peptide Institute, University of Alberta, Edmonton.
ARC	Alberta Research Council.
ATX-a	Anatoxin-a secondary, bicyclic amine alkaloid neurotoxin.
BMAA	β-N-methylamino-L-alanine amino acid neurotoxin
DWQ	Drinking water quality.
Euphotic Zone	The depth of the water column in a lake or reservoir that is exposed to sunlight intensity sufficient for supporting photosynthesis. It extends from the water's surface (air-water interface) to a depth where light intensity is equal to 1 percent of that at the surface.
Eutrophic	Elevated trophic state. A lake or reservoir with high productivity (high concentrations of nutrients and resulting high algal/plant growth).
GC-MS	Gas chromatography linked mass spectrometry
HPLC	High performance liquid chromatography
Hypereutrophic	Highly elevated trophic state productivity. A lake or reservoir with very high productivity (very high concentrations of nutrients and resulting very high algal/plant growth).
IC_{50}	The concentration of a substance that inhibits a biological response or process (i.e., enzyme activity, cell growth) by 50%.
LC-MS/MS	Liquid chromatography linked tandem mass spectrometry.

LD_{50}	The dose of a substance that causes mortality to 50% of the test organisms.
LTLN	Alberta Environment's Long-term Lake Network
MAC	Maximum acceptable concentration.
MCLR	Microcystin analogue with (L)eucine and a(R)ginine in variable amino acid positions 2 and 4.
MCYST	Microcystin cyclic peptide hepato- (liver) toxin.
Mesotrophic	Moderate trophic state. A lake or reservoir with moderate productivity (moderate concentrations of nutrients and resulting moderate algal/plant growth).
Metalimnetic Bloom	An accumulation of buoyancy-regulating cyanobacteria, notably *Planktothrix sp.*, within a distinct depth stratum at or near the metalimnion (transition between the well-mixed upper illuminated layer and deep, isolated waters) of a stratified lake or reservoir.
NOAEL	No-observed-adverse-effects-level: denotes the level of exposure of a test organism, at which there is no biologically or statistically significant increase in the frequency or severity of any adverse effects in the exposed population when compared to its appropriate control.
OATPs	Organic anion transporter polypeptides are transmembrane proteins expressed in various organs/tissues that function in the uptake of compounds including enzymes, drugs and toxins.
Oligotrophic	Low trophic state. A lake or reservoir with low productivity (low concentrations of nutrients and resulting low algal/plant growth).
PPI	Protein phosphatase enzyme inhibition.
PPLMP	Alberta Environment's Provincial Parks Lake Monitoring Program.
TDI	Tolerable daily intake: an estimate of the intake of a substance over a lifetime that is considered acceptable without appreciable health risk.
Trophic State	The overall level of biological productivity (or fertility) of a lake and is usually defined by concentrations of key nutrients (primarily phosphorus) and algae that are present
WHO	The World Health Organization.

ACKNOWLEDGEMENTS

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1.0 INTRODUCTION

1.1 Cyanobacteria Occurrence and Toxicity

Eutrophic (nutrient-rich) lakes and reservoirs in Alberta often experience blooms of cyanobacteria during summer and early fall. It is well known that some common species of cyanobacteria produce potent liver and/or nerve toxins. Microcystins (MCYSTs) are the most common group of toxins produced by cyanobacteria and exert toxicity by severely damaging the structure and function of the liver representing a significant threat to humans (Dunn, 1996), pets and livestock (Gurney and Jones, 1997). They are tumor promoters and chronic exposure to MCYSTs has been linked to high incidence of primary liver (hepatocellular carcinoma) and colorectal cancers in rural human populations around the world (Zhou *et al.*, 2002). Recent studies have also shown MCYSTs to induce oxidative DNA damage in liver cell isolates suggesting the toxin can initiate cancer (Žegura *et al.*, 2003).

Several bloom-forming species of cyanobacteria (including *Microcystis aeruginosa*, *M. flos-aquae*, *M. wesenbergii*, *Anabaena flos-aquae*, *A. circinalis*, *A. lemmermannii*, *Planktothrix agardhii* and *P. rubescens*) produce MCYSTs. To date, more than 80 toxin analogues of MCYST have been isolated and described globally. They are small monocyclic peptides composed of seven amino acids including an amino acid residue, abbreviated Adda, unique to MCYSTs (and Nodularin from marine cyanobacteria) and critical for toxicity (Figure 1). The 20 or so primary MCYST analogues differ with respect to variable L-amino acids at positions **2** and **4** – denoted by X and Y, respectively. Of these, microcystin-LR (MCLR), which possesses leucine (L) and arginine (R) at positions **2** and **4**, was one of the first discovered and consequently is the most studied congener (Table 1). Alterations and substitutions of other constituent amino acids, including demethylation of D-MeAsp and/or Mdha (positions **3** and **7**, respectively), methyl esterification of D-Glu (position **6**) and geometric isomerization of Adda (position **5**) result in numerous additional toxic and non-toxic congeners (reviewed in Zurawell, 2001).

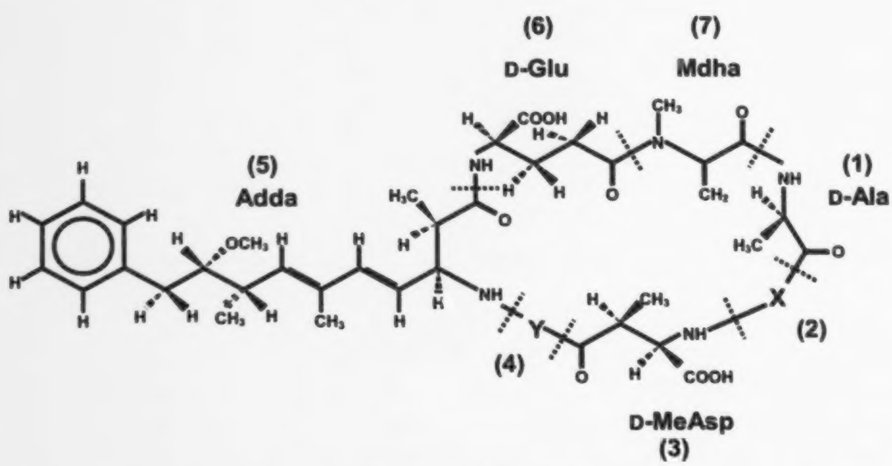

Figure 1 **Generalized chemical structure of MCYST: where position (1) is D-Alanine; (2) X is a variable L-amino acid; (3) is D-erythro-β-methylaspartic acid; (4) Y is another variable L-amino acid; (5) is Adda, (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid; (6) is D-Glutamic acid and (7) is N-methyldehydroalanine (adapted from Zurawell, 2001).**

Table 1 Structure and comparative toxicity of 20 primary microcystin analogues based on interperitoneal LD_{50} in mice (µg/kg); ND, not determined; Aba, L-aminoisobutyric acid; Hil, L-homoisoleucine; Hty, L-homotyrosine; M(O), methionine S-oxide; (H4)Tyr, 1,2,3,4-tetrahydrotyrosine (adapted from Zurawell, 2001).

Analogue	Structure	Toxicity
Microcystin-AR	cyclo (-D-Ala-**L-Ala**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	250
Microcystin-FR	cyclo (-D-Ala-**L-Phe**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	250
Microcystin-HilR	cyclo (-D-Ala-**L-Hil**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	100
Microcystin-HtyR	cyclo (-D-Ala-**L-Hty**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	80-100
Microcystin-LA	cyclo (-D-Ala-**L-Leu**-D-MeAsp-**L-Ala**-Adda-D-Glu-Mdha-)	50
Microcystin-LAba	cyclo (-D-Ala-**L-Leu**-D-MeAsp-**L-Aba**-Adda-D-Glu-Mdha-)	ND
Microcystin-LF	cyclo (-D-Ala-**L-Leu**-D-MeAsp-**L-Phe**-Adda-D-Glu-Mdha-)	ND
Microcystin-LL	cyclo (-D-Ala-**L-Leu**-D-MeAsp-**L-Leu**-Adda-D-Glu-Mdha-)	ND
Microcystin-LM	cyclo (-D-Ala-**L-Leu**-D-MeAsp-**L-Met**-Adda-D-Glu-Mdha-)	ND
Microcystin-LR	**cyclo (-D-Ala-L-Leu-D-MeAsp-L-Arg-Adda-D-Glu-Mdha-)**	**50**
Microcystin-LV	cyclo (-D-Ala-**L-Leu**-D-MeAsp-**L-Val**-Adda-D-Glu-Mdha-)	ND
Microcystin-LY	cyclo (-D-Ala-**L-Leu**-D-MeAsp-**L-Tyr**-Adda-D-Glu-Mdha-)	90
Microcystin-M(O)R	cyclo (-D-Ala-**L-Met(O)**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	700-800
Microcystin-RA	cyclo (-D-Ala-**L-Arg**-D-MeAsp-**L-Ala**-Adda-D-Glu-Mdha-)	ND
Microcystin-RR	cyclo (-D-Ala-**L-Arg**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	500-800
Microcystin-WR	cyclo (-D-Ala-**L-Try**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	150-200
Microcystin-YA	cyclo (-D-Ala-**L-Tyr**-D-MeAsp-**L-Ala**-Adda-D-Glu-Mdha-)	60-70
Microcystin-YM(O)	cyclo (-D-Ala-**L-Tyr**-D-MeAsp-**L-Met(O)**-Adda-D-Glu-Mdha-)	56-110
Microcystin-YR	cyclo (-D-Ala-**L-Tyr**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	150-200
Microcystin-(H4)YR	cyclo (-D-Ala-**L-(H4)Tyr**-D-MeAsp-**L-Arg**-Adda-D-Glu-Mdha-)	ND

In addition to MCYSTs, some species of cyanobacteria including *A. flos-aquae* and *A. spiroides*, produce a nerve toxin called anatoxin-a (ATX-a). This is a highly potent toxin that interferes with nervous system function by disrupting the normal propagation of nerve impulses from neurons to muscles, with the potential to cause paralysis and death via respiratory failure and suffocation in animals. Like MCYST, the occurrence of ATX-a is a global concern because it has been linked to the deaths of livestock, pets and wildlife around the world, including the Canadian prairies (Zurawell, 2001). In all previous cases within Alberta, the assumption of ATX-a poisoning has largely been based on the presence of cyanobacteria and symptoms in affected animals. The actual presence of ATX-a in these instances has not been documented prior to the work reported here.

Beta-*N*-methylamino-L-alanine (BMAA) has recently been identified in terrestrial, marine and freshwater cyanobacteria (Cox *et al.*, 2005). The amino acid has been linked to Alzheimer's-like neurodegeneration in human populations and is a possible causative agent of Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex (ALS/PDC; Kurland and Mulder, 1954; Spencer *et al.*, 1987). Additionally, it has been isolated and identified in brain tissue of Canadians who have succumbed to Alzheimer's disease, though the source of BMAA in these instances remains unknown (Murch *et al.*, 2004a). Unlike MCYST or ATX-a, which elicit immediate acute toxicity, it appears BMAA can accumulate in tissues and bio-magnify through the food chain. It is hypothesized that accumulated BMAA is slowly released over years as proteins are naturally metabolized, causing recurrent neurological damage (Murch *et al.*, 2004b). Note that the proposed mechanism requires further assessment and an appropriate animal model relevant to humans must be demonstrated to validate the role of this putative neurotoxin. The question exists whether BMAA occurs in cyanobacteria inhabiting Alberta's surface waters.

1.2 Drinking Water Guidelines

Global concern over the implications of MCLR to human health peaked during the late-1990's in the wake of human tragedies in Caruaru, Brazil in 1996 – 49 kidney dialysis patients died from acute liver failure following microcystin exposure through dialysis treatment (Pouria *et al.*, 1998). Following the lead of the World Health Organization (WHO), Health Canada sought to develop a drinking water quality (DWQ) guideline for MCLR, which was derived in accordance with the WHO's approach. First, a tolerable daily intake (TDI) for MCLR was calculated based on: (1) an estimated no-observed-adverse-effects-level (*NOAEL*) following chronic exposure of mice to purified MCLR (study by Fawell *et al.*, 1994) and (2) provision for uncertainties (*UF*) due to intra- and interspecies variation and a less-than-lifetime study (see Equation 1).

Equation (1):

$$\mathrm{TDI} = \frac{NOAEL}{UF} = \frac{40\ \mu\mathrm{g/kg\ bw/d}}{1000} = 0.04\ \mu\mathrm{g/kg\ bw/d}$$

where:

- *NOAEL* = a no-observed-adverse effects level of 40 µg/kg body weight per day derived from observed liver changes during a 13-week mouse study conducted by Fawell *et al.* (1994).
- *UF* = an uncertainty factor of 1000; the product of applying a 10× factor each for: (1) intraspecies variation, (2) interspecies variation and (3) a less-than-lifetime study.

Next, the TDI was multiplied with the body weight of an average adult in Canada (*bw*) and with the proportion of total toxin exposure attributed to the consumption of drinking water (*P*). This product, divided by the average volume of drinking water (*L*) consumed by an adult in Canada is equal to a maximum acceptable concentration (MAC) of 1.5 µg/L for total MCLR (applies to the sum of intra- and extracellular MCLR) in finished drinking water (see Equation 2; Health Canada, 2002). While the approach parallels that of the WHO, the derivation of the Canadian DWQ guideline differs with respect to values considered for: (1) the average body weight of an adult (70 kg Canadian vs. 60 kg WHO) and (2) the average daily consumption rate of drinking water (1.5 L/d Canadian vs. 2 L/d WHO); and using the values specified by the WHO yields a lower provisional guideline of 1.0 µg/L MCLR.

Equation (2):

$$\mathrm{MAC} = \frac{TDI \times bw \times P}{L} = \frac{0.04\ \mu\mathrm{g/kg\ bw/d} \times 70\ \mathrm{kg\ bw} \times 0.8}{1.5\ \mathrm{L/d}} \approx 1.5\ \mu\mathrm{g/L}$$

where:

- *TDI* = the calculated tolerable daily intake of 0.04 µg/kg bw per day in equation 1 (above).
- *bw* = the average body weight of an adult in Canada; 70 kg is typically used. *Note: WHO used 60 kg - the international unit adult average weight.*
- *P* = the proportion of total toxin intake (exposure) attributed to ingestion of drinking water. Drinking water consumption is felt to be the major route of microcystin exposure, hence 80% was used.
- *L* = the average daily consumption of drinking water for an adult in Canada is 1.5 L/d. *Note: WHO used 2 L/d average daily consumption of drinking water.*

Note, however, that neither the WHO nor Health Canada have accounted for total MCYST, only MCLR in their derived MACs. In fact, the WHO guideline is stated as being 'provisional' citing insufficient toxicological data on other analogues to warrant inclusion (at the time). In contrast, Australia developed a guideline for total MCYST based on MCLR "toxicity equivalents" (i.e., to infer concentration of MCYST in a sample by determining concentration of purified MCLR required to generate an equivalent toxic response). Their rationale included the fact that blooms of *M. aeruginosa* (the most common bloom-forming cyanobacterium in Australia) contain several to many analogues (more than 20 in some cases) in an individual sample and that cumulative toxicity of MCYSTs represents the potential threat to human health via drinking water consumption (Nicholson and Burch, 2001).

At the time of (DWQ) guideline development, the ability of MCLR to promote tumour growth was only suspected. Thus, provisions for this characteristic were not incorporated in the uncertainty factor of the TDI calculation. Subsequent studies not only confirm the ability of MCYSTs to promote tumour growth, but have established a link between toxin exposure and primary liver and colorectal cancers in human populations (Nishiwaki-Matsushima *et al.*, 1992; Humpage *et al.*, 2000; Zhou *et al.*, 2002).

1.3 Monitoring Program and Objectives

Research into the occurrence of MCYSTs in Alberta's lakes and reservoirs began in the late 1980's. However, most of these studies focused on MCLR and as a result less information exists on the prevalence of other congeners – some of which possess potency similar to that of MCLR. In 2005, AENV incorporated sampling and analysis of water for total MCYST concentration into its Provincial Lake monitoring programs including the Long-term Lake Network (LTLN) and the priority-one lakes of the Provincial Parks Lake Monitoring Program (PPLMP). In addition, joint research between AENV and the Alberta Research Council (ARC, Vegreville) permitted the analysis of total microcystin in 100 samples collected from priority-two lakes of the PPLMP and lakes sampled by the Alberta Lake Management Society's (ALMS) Lake Monitoring program – Lakewatch. MCYST analysis was added to all lake and reservoir monitoring programs province-wide in 2006 and continued through 2008. Provincial monitoring programs included the LTLN, PPLMP, Southern AB Lakes and Reservoirs Program, Central Recreational Lake Monitoring Program, Elk Island National Park Program and ALMS' Lakewatch Program.

Samples collected from lake monitoring programs in 2005 were analyzed for specific MCYST analogues including MCLR, MCYR and MCRR. This work was revisited in 2007 and a set of 44 samples were analyzed for 5 specific analogues including: MCLR, MCYR, MCRR, MCLF and MCLW. Analysis for ATX-a was also conducted on 66 water samples in 2005 and 10 additional samples collected in 2006. Research into the detection and quantification of BMAA in surface water samples was initiated in 2005 by AENV in partnership with the Alberta Peptide Institute (API) at the University of Alberta. Methods were developed to isolate and detect BMAA in routine surface water samples. Method development for the detection of BMAA continued in 2007 when a new research partnership was formed with The Alberta Centre for Toxicology, University of Calgary (ACFT).

This report presents status and findings of the cyanotoxin monitoring program from 2005 – 2008.

2.0 METHODS

Euphotic (depth) integrated composite water samples were collected for chemical (total MCYST, MCYST analogue and ATX-a concentrations) and biological (phytoplankton species identification and enumeration) analyses. Euphotic integrated samples were, in most cases, collected from 10 sites on a lake/reservoir basin and combined, together, to form a composite water sample as specified in AENV (2006). Sites were selected to encompass both near- (shallow near shoreline) and off-shore (open-water) areas and influences of embayment.

Cyanobacteria are largely planktonic and can accumulate with wind and wave action resulting in significantly greater population density and toxin concentrations along leeward shorelines compared to those upwind (i.e., horizontal heterogeneity). Moreover, many cyanobacteria possess specialized gas vesicles that aid in regulating buoyancy allowing them to migrate vertically within the water column to depths of optimal light intensity and spectrum and nutrient concentrations. They produce excess gas vesicles during periods of wind-induced water-column mixing to counter the downward drag of water currents. When winds cease, cyanobacteria become over-buoyant and accumulate near the water's surface producing what is commonly called a bloom and appearing as a visible paint-like scum.

Buoyancy regulation and blooms result in significant vertical heterogeneity in cyanobacteria distribution. The euphotic (depth) integrated composite water samples accounts for vertical and horizontal spatial heterogeneity that may exist in a water body in an attempt to represent average whole-lake estimates of cyanobacteria density and toxin content. Note that this method of sampling does not determine peak cyanobacterial densities and toxin concentrations that occur near the surface and immediately adjacent to leeward shoreline areas; surface blooms may contain MCYSTs at concentrations 2 or even 3 orders of magnitude higher than open water estimates convey.

During the 4 year period, samples were submitted to several laboratories depending on analyte of interest and laboratory capability. Sample handling and analytical methodology are detailed in Appendix I. Efforts were made to address aspects of quality assurance and control (QA/QC) pertaining primarily to sample analyses. Results are detailed in Appendix IV.

3.0 RESULTS AND DISCUSSION

3.1 Microcystin Monitoring 2005 - 2008

2005 Results

During the 2005 open water season (May through October), a total of 164 euphotic depth integrated composite samples were collected and analyzed for total MCYST concentration via PPI assay (detection limit of 0.1 µg MCLR eq./L). Of these samples, 140 were collected from 34 natural lakes and 24 were collected from 6 reservoirs across the province (Table 2). Waterbodies were chosen simply based on their inclusion in an existing monitoring program and not on their trophic status (Appendix II, Table A1) or the historical occurrence of blooms within them. Surface waters ranged in trophic status from unproductive and least likely to experience surface blooms of cyanobacteria (e.g., Gregg and Jarvis lakes; Upper and Lower Kananaskis reservoirs) to hypereutrophic, bloom-impacted lakes (e.g., Baptiste and Sturgeon lakes). Raw data are presented in Appendix V, Table A7.

Microcystin was detected in 79 (48%) of the 164 samples (Table 2; Figure 2). The majority (80%) of these, contained MCYST at concentrations up to 0.5 µg/L and approximately 1% (1 sample) contained greater than 1.5 µg/L of MCYST – the current DWQ guideline for MCLR (Figure 2). Samples collected in 2005 never contained MCYST in excess of the proposed RWQ guideline of 20 µg/L (Table 2). These findings are consistent with previous studies that indicated the majority of surface waters contain low (i.e., up to 0.5 µg/L) concentrations of MCYST (Zurawell, 2002). Concentrations can be elevated during August and September and may exceed 10 µg/L (Kotak and Zurawell, 2006). Concentrations were less than 2 µg/L during the 2005 program (Appendix III, Figure A1). It is important to note these reported volumetric concentrations are from depth integrated water samples collected from the euphotic zone and not concentrated surface bloom samples. Grab samples from the surface during severe blooms can yield considerably higher toxin concentrations (i.e., > 5000 µg/L; Kotak and Zurawell, 2006).

Table 2 Summary of euphotic integrated composite samples collected during the open-water (May through October) seasons 2005 through 2008.

	2005	2006	2007	2008
Total # of samples collected	164	182	164	220
# (%) of samples with MCYST not detected	85 (52%)	85 (47%)	40 (24%)	94 (43%)
# (%) of samples with MCYST detected (≥0.1 ug/L)	79 (48%)	97 (53%)	124 (76%)	126 (57%)
# (%) of samples over DW guideline (1.5 ug/L)	1 (1%)	11 (11%)	39 (32%)	16 (13%)
# (%) of samples over proposed Rec. guideline (20 ug/L)	0 (0%)	0 (0%)	2 (2%)	0 (0%)
Total # of lake samples	140	156	144	197
Total # of reservoir samples	24	26	20	23
# of lake basins sampled	34	42	43	45
# of reservoir basins sampled	6	7	8	7

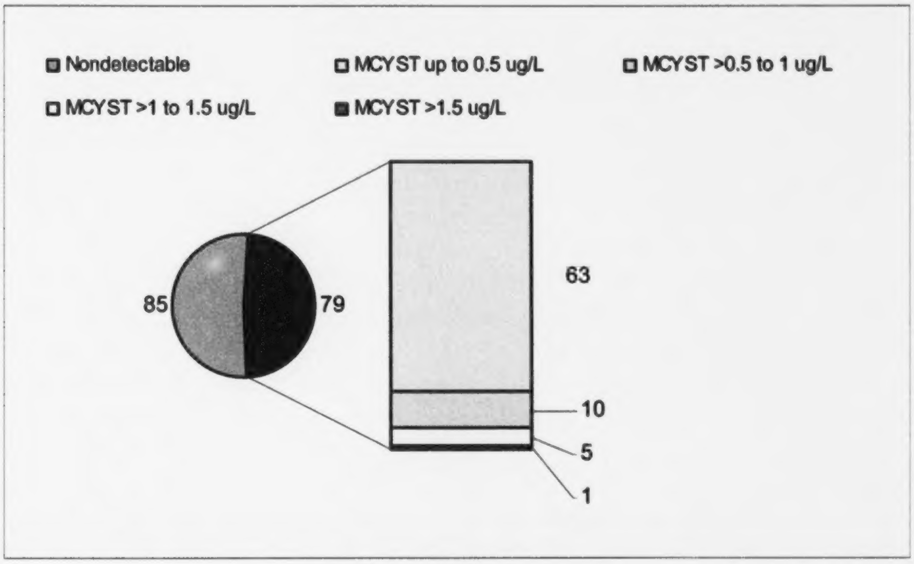

Figure 2 Number of surface water samples from 2005 with nondetectable (85 samples; blue portion of pie chart) vs. detectable (79 samples; red portion of pie chart) levels of MCYST. The bar summarizes the number of samples with MCYST at various incremental concentrations.

During the 2005 monitoring program, MCYST was detected on 1 occasion in 26 (76%) of the 34 lakes and in 4 (67%) of the 6 reservoirs. Toxin was not detected in Beauvais, Elkwater, Fishing, Fork, Gregg, Gregoire, Island, and Jarvis lakes or in Upper and Lower Kananaskis reservoirs in 2005. The fact that 75% of the waterbodies sampled during the 2005 open water season contained detectable levels of MCYST on at least 1 occasion (see Section 3.5 below, Figure 6) suggests the toxin is prevalent in Alberta's surface waters. Microcystin was not detected in several eutrophic lakes (Fork, Gregoire and Island lakes) and one bloom-prone hypereutrophic lake (Fishing Lake) during this period (Appendix II, Table A1). Toxin was detected in a number of mesotrophic surface waters including Crimson, Dillberry, Frog, Garnier (North), Hilda, Miquelon, Whitefish and Wolf lakes and Spruce Coulee and Newell reservoirs (Appendix II, Table A1; Figure 6).

2006 Results

A total of 182 composite samples were collected and analyzed for total MCYST concentration during the 2006 season. Of these samples, 156 were collected from 42 natural lakes and 26 from 7 reservoirs across the province (Table 2). As in 2005, these surface waters were chosen simply based on their inclusion in an existing monitoring program and not on their level of fertility (trophic status; Appendix II, Table A2) or the historical occurrence of blooms within them. The surface waters range in trophic status from oligotrophic (e.g., Gregg and Jarvis lakes; Upper and Lower Kananaskis and Gleniffer reservoirs) to hypereutrophic, bloom-impacted lakes (e.g., Baptiste, Steele and Sandy Lakes). Raw data are presented in Appendix V, Table A8.

Microcystin was detected in 97 (53%) of the 182 samples (Figure 3). As in 2005, the majority (65%) of these, contained low MCYST concentrations (i.e., up to 0.5 µg/L); 15% contained greater than 0.5 and up to 1.0 µg/L of MCYST; 8% contained greater than 1.0 and up to 1.5 µg/L of MCYST; and approximately 11% (11 of the 97 samples) contained greater than 1.5 µg/L of MCYST. In general, these findings were consistent with those of 2005 and previous studies

indicating the majority of surface waters contain low (i.e., ≤ 0.5 μg/L) concentrations of MCYST, on a whole-lake average basis. Compared to the previous year, however, a greater percentage of samples collected in 2006 contained concentrations of MCYST in excess of the DWQ guideline of 1.5 μg/L (11% in 2006 compared to 1% in 2005). Also, peak MCYST concentrations were greater in 2006 than in the previous year, as 4 samples (2 from Cooking Lake and 1 each from Red Deer and Pigeon lakes) exceeded 2 μg/L (Appendix III, Figure A2). As in 2005, no samples contained MCYST in excess of the proposed RWQ guideline of 20 μg/L. Once again it is important to note these concentrations are from depth integrated, multiple-site, composite water samples and not from surface concentrated bloom samples (such as those regularly occurring along beaches), which yield considerably more toxin.

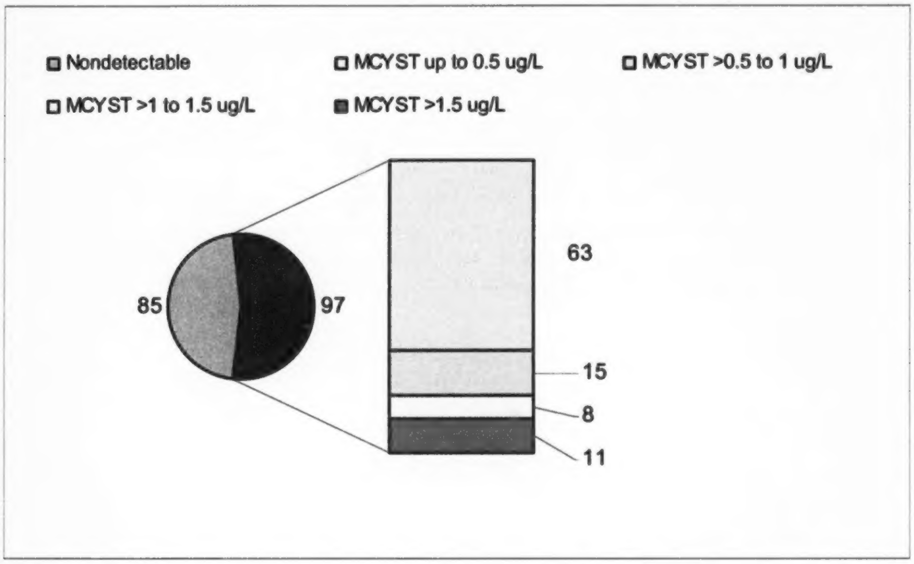

Figure 3 **Number of surface water samples from 2006 with nondetectable (85 samples; blue portion of pie chart) vs. detectable (97 samples; red portion of pie chart) levels of MCYST. The bar summarizes the number of samples with MCYST at various incremental concentrations.**

In 2006, MCYST was detected on at least 1 occasion in 34 (81%) of the 42 lakes and 3 (43%) of the 7 reservoirs. Toxin was not detected in Beauvais, Clear (Barns), Ethel, Frog, Moose, Sylvan, Tucker and Wolf lakes or Moonshine, Newell, Lower Kananaskis and Spruce Coulee reservoirs in 2006. Overall, 76% of waterbodies sampled in 2006 contained detectable MCYST on at least one occasion. This finding is consistent with the previous year (75% in 2005) and indicates that MCYST is prevalent in Alberta's lakes and reservoirs.

Toxin was detected in a number of oligotrophic (Gregg and Jarvis lakes and Upper Kananaskis and Gleniffer reservoirs) and mesotrophic (Beartrap, Crimson, Dillberry, Elkwater, Hilda, Miquelon and Muriel lakes) waterbodies, while not being detected in several eutrophic (Beauvais, Moose and Tucker lakes and Moonshine reservoir) and hypereutrophic, Clear (Barns) Lake, systems (Appendix II, Table A2; Figure 6).

2007 Results

A total of 164 composite samples were collected and analyzed for total MCYST concentration in 2007 - 144 samples from 43 lakes and 20 from 8 reservoirs province-wide (Table 2). As in the previous 2 years of monitoring, the surface waters ranged in trophic status from oligotrophic to

hypereutrophic (Appendix II, Table A3). MCYST was detected in 124 (76%) of the 164 samples (Figure 4). In contrast to the previous seasons, far fewer samples collected in 2007 contained low (i.e., up to 0.5 µg/L) concentrations of MCYST – 47% of samples in 2007 vs. 80% and 65% in 2005 and 2006, respectively. The percentages of samples containing moderately-low (i.e., greater than 0.5 and up to 1.0 µg/L) and moderate (greater than 1.0 and up to 1.5 µg/L) MCYST concentrations were 10% and 11%, respectively, and were similar to that observed in 2005 and 2006. The most striking distinctions in observed toxin levels in 2007 were the high proportion (30%) of MCYST-positive samples exceeding 1.5 µg/L of MCYST (the DWQ guideline) and the presence of 2 (sequential) samples from George Lake exceeding the newly proposed RWQ guideline of 20 µg/L. Concentrations in George Lake peaked on August 01, 2007 at nearly 46 µg/L making this the highest recorded whole lake composite MCYST levels over the 4 years of monitoring. A sequential sample taken from the lake on August 23, 2007 contained nearly 22 µg/L of MCYST and notably near the proposed RWQ guideline well into September (19.3 µg/L on September 18, 2007).

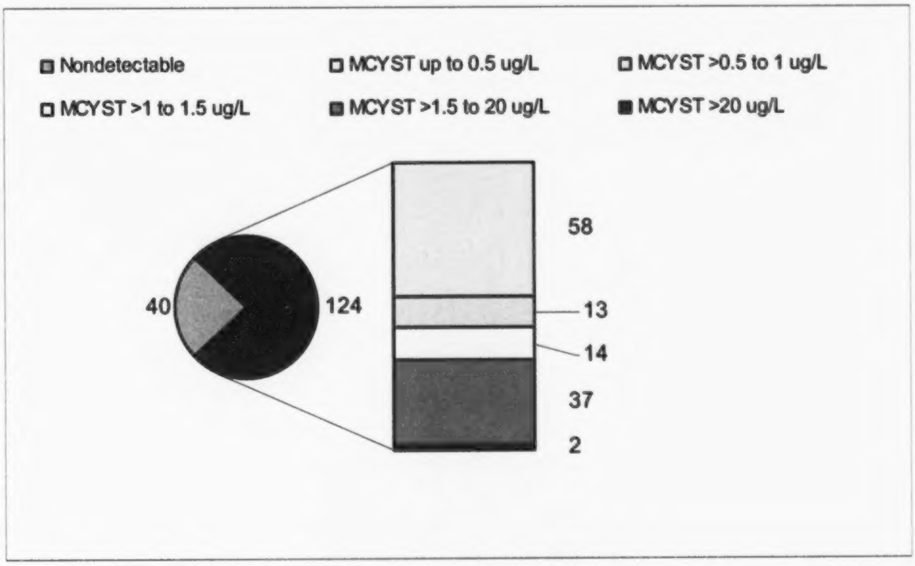

Figure 4 **Number of surface water samples from 2007 with nondetectable (40 samples; blue portion of pie chart) vs. detectable (124 samples; red portion of pie chart) levels of MCYST. The bar summarizes the number of samples with MCYST at various incremental concentrations.**

Other lakes also appeared to have experienced severe blooms of toxic (MCYST-containing) cyanobacteria in 2007. A total of 15 samples collected from 9 lake basins (Baptiste North and South basins, Clear, Cooking, George, Saskatoon, Steele, Thunder and Winagami lakes) contained levels of MCYST near or in excess of 10 µg/L (Appendix III, Figure A3). Raw data are presented in Appendix V, Table A9.

Toxin was detected on at least 1 occasion in all 43 (100%) of the lakes and 6 of the 8 reservoirs. Thus overall, 96% of waterbodies sampled in 2007 contained detectable MCYST on at least one occasion signifying an increase of about 20% in prevalence over previous years (Figure 6).

Toxin was not detected in Newell Lake or Twin Valley reservoirs in 2007, but this may be an artifact of sampling frequency as sampling occurred on a single date foı each. Given additional opportunity to collect samples over the entire open-water season, it is likely that toxin could have

been detected in these two reservoirs bringing MCYST prevalence to 100% of the systems monitored. See section *Year-to-Year Microcystin Variability* below for further discussion.

2008 Results

In 2008, a total of 220 samples were collected for total MCYST analysis – 197 samples from 45 lake basins and 23 samples from 7 reservoirs across the province (Table 2; Appendix II, Table A4). Toxin was detected in 126 (57%) of the 220 samples (Figure 5). Similar to the 2005 and 2006 seasons, the majority (69%) of these contained low MCYST concentrations (up to 0.5 µg/L); 11% contained greater than 0.5 and up to 1.0 µg/L of MCYST; 7% contained greater than 1.0 and up to 1.5 µg/L of MCYST. And like that observed in 2006, a portion (13%) of samples contained greater than 1.5 µg/L of MCYST (the DWQ guideline). Unlike 2007, no samples contained MCYST in excess of the proposed RWQ guideline of 20 µg/L – though several (3) from Oster and Sandy lakes exceeded 10 µg/L (Appendix III, Figure A4). Raw data are presented in Appendix V, Table A10.

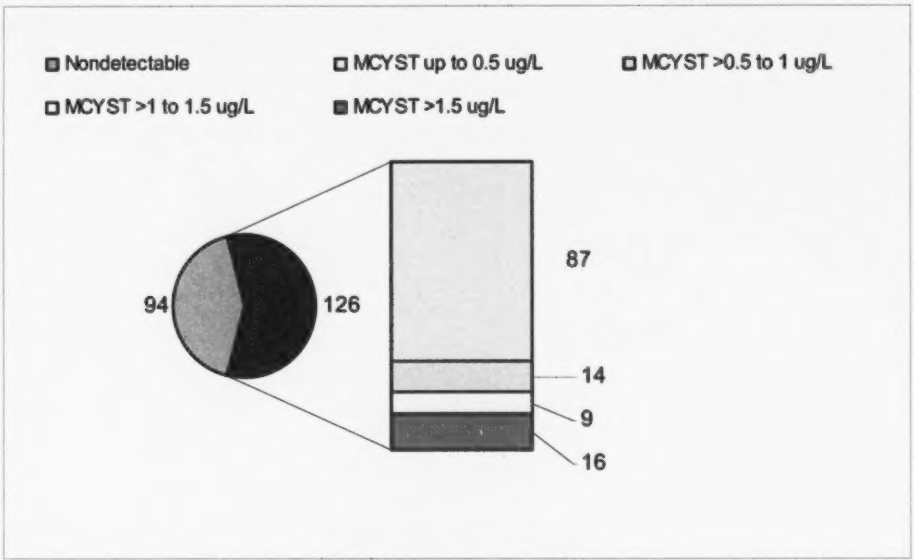

Figure 5 Number of surface water samples from 2008 with nondetectable (94 samples; blue portion of pie chart) vs. detectable (126 samples; red portion of pie chart) levels of MCYST. The bar summarizes the number of samples with MCYST at various incremental concentrations.

MCYST was detected on at least 1 occasion in 40 (89%) of the 45 lakes and 4 (57%) of the 7 reservoirs. Toxin was not detected in Beauvais, Elkwater, Gregg, Jarvis or Sylvan lakes, nor did it occur once in Upper and Lower Kananaskis and Reesor reservoirs in 2008. Overall, 85% of waterbodies sampled in 2008 contained detectable MCYST on at least one occasion – down 10% in prevalence from 2007, yet 10% greater than in both 2005 and 2006 (Figure 6). Also, MCYST was detected in Newell and Twin Valley reservoirs in 2008 – the only two locations with non-detectable levels in 2007.

Microcystin Prevalence 2005 - 2008 Summary Data

Microcystin was prevalent during the 4 years of monitoring. Toxin was detected on at least one sampling occasion in 75% (2005) to 96% (2007) of the lakes and reservoirs sampled in a given

year (Figure 6); and was generally more prevalent in fertile (i.e., hypereutrophic and eutrophic) waterbodies than in less productive systems. However, toxin was common (i.e., occurring in > 50% of waterbodies) in mesotrophic waters in all 4 years and in oligotrophic systems in 2006 and 2007 (Figure 6).

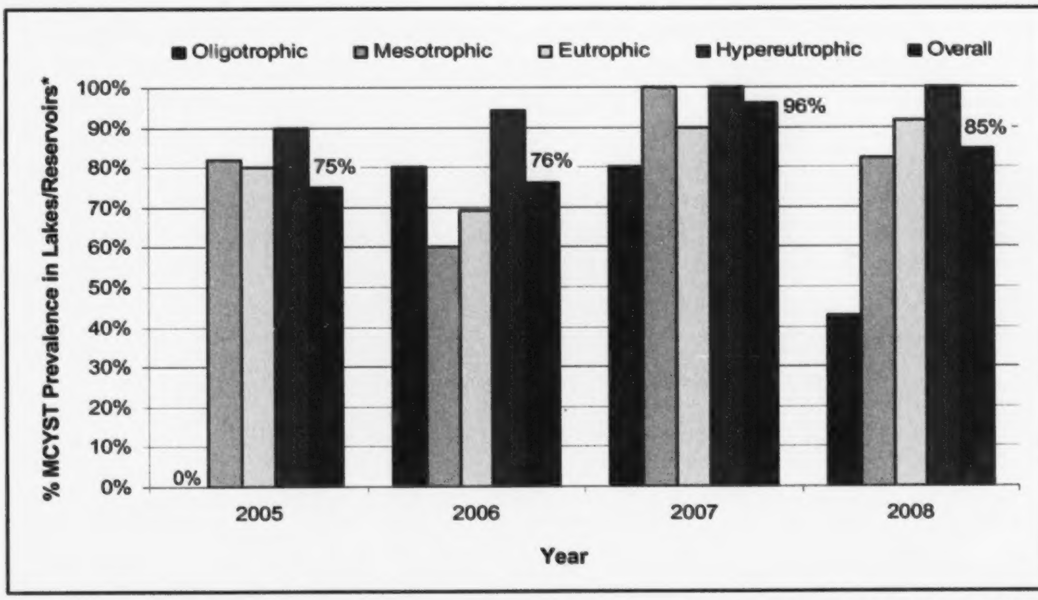

Figure 6 Prevalence of MCYST in lakes/reservoirs of varying trophic status from 2005-2008. *MCYST prevalence is the percentage of lakes/reservoirs containing detectable toxin (≥ 0.1 µg MCLReq./L) on at least one sampling occasion.

Microcystin in Low Nutrient Lakes

Oligotrophic and mesotrophic lakes generally do not support large populations of phytoplankton. As a result, surface blooms of cyanobacteria are rare in these environments. It is well established that less fertile, stratified lakes and reservoirs, experience periodic accumulations of cyanobacteria at depths several meters below the surface nearer the metalimnion (i.e., transition between the upper well-mixed and illuminated epilimnion and the deeper, isolated cold water hypolimnion). Metalimnetic blooms occur when cyanobacteria, notably *Planktothrix (Oscillatoria) sp.*, congregate within a distinct 1 to 2 m thick stratum at depth. These so-called 'metalimnetic ecostrategists' possess phycobiliproteins – water soluble photosynthetic pigments (in addition to chlorophyll) that efficiently intercept the breadth of the light spectrum (400-700 nm). Some *Planktothrix* (particularly *rubescens*) perform photosynthesis at very low light and grow at light intensities 1-5% of surface irradiance. They are also able to effectively regulate buoyancy in order to remain at these depths of optimal light.

Evidence from past field observations and other research in Alberta (Zurawell, unpublished) and elsewhere (e.g., in Finland by Lindholm and Meriluoto, 1991), indicate metalimnetic blooms occur in meso- and oligotrophic waters and often contain MCYST. This means even those lakes suffering little human impact, may occasionally become toxic. Sampling protocols employed for monitoring ensured water was collected from the entire euphotic zone. Toxin-producing metalimnetic cyanobacteria, if present, would have been collected and could account for the MCYST periodically contained within samples from meso- and oligotrophic lakes and reservoirs during the 4 years of study.

Although not an initial objective of the monitoring program, provisions were made from 2006 onward to collect evidence indicating the presence of MCYST-producing metalimnetic cyanobacteria. Supersaturating (near 100%) oxygen conditions isolated at depth can denote the presence of photosynthetically active metalimnetic cyanobacteria. Field technicians were instructed to collect discrete samples for total MCYST analysis and phytoplankton identification should they encounter supersaturating conditions at depth. On one occasion supersaturating conditions were noted at 8-m depth (as determined by a Hydrolab oxygen sensor) in oligotrophic Jarvis Lake on September 4, 2006. A discrete sample collected from 8-m depth stratum contained 0.16 μg/L of MCYST. Unfortunately, a sample for the identification of phytoplankton was not collected and the assumption of the existence of metalimnetic cyanobacteria remains somewhat speculative. Notably, toxin was not detected (i.e., <0.1 μg MCLReq./L) in the euphotic integrated composite sample collected from Jarvis Lake on that date. The discrepancy could simply be due to dilution of MCYST within a discrete depth by toxin-free overlying water – all collected as part of the euphotic integrated sample protocol.

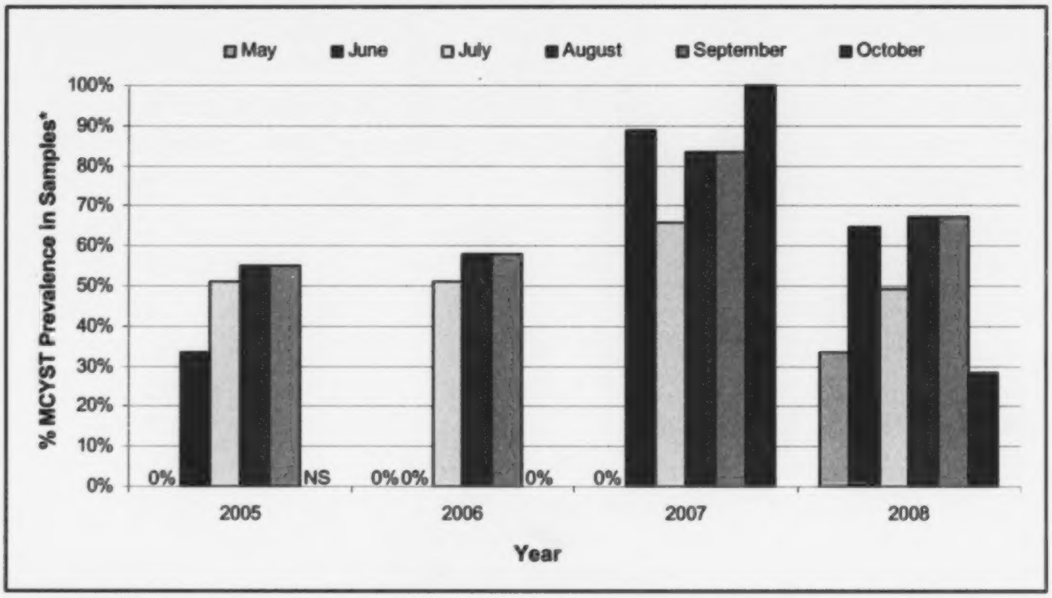

Figure 7 Prevalence of MCYST in surface water samples grouped by sampling month (2005-2008). *MCYST prevalence is the percentage of samples containing detectable toxin (≥ 0.1 μg MCLReq./L).

Microcystin was detected in 48% (2005) to 76% (2007) of the samples collected in a given year (Table 2). As anticipated, MCYST was generally more prevalent during the months of August and September (Figure 7). Cyanobacterial blooms in Alberta lakes and reservoirs usually occur – and are more severe – from mid-summer through early fall as water column stability required for surface bloom formation is greatest during this period (Figure 7).

Year-to-Year Microcystin Variability

Our data suggest that the presence of MCYST in a given water body is highly variable year to year. For instance, toxin was not detected in Elkwater, Fishing, Gregg, Gregoire, Island, or Jarvis lakes or Upper Kananaskis reservoir in 2005, but was detected at least once in each during 2006. In contrast, Moose and Wolf lakes and three reservoirs, Moonshine, Newell and Spruce Coulee, contained MCYST at least once in 2005, but did not contain toxin in 2006.

In some instances, low sampling frequency may explain year-to-year disparity in the presence/absence of MCYST, as only one sample may have been collected in a given year. Considering the dynamic nature of phytoplankton communities, a single sampling event over an open water season is not sufficient to capture the presence of toxin-producing cyanobacteria. This is especially true for less productive waters, as metalimnetic cyanobacteria reach high densities for short periods of time. This may have been the case for Gregg and Jarvis lakes, as both were only sampled once each in 2005 (MCYST not detected), yet did contain MCYST in 2006 when they were sampled four times each. Similarly, toxin was not detected in each single sample collected in either 2005 or 2006 from Beauvais Lake. However, MCYST was detected on 2 of 4 occasions in Beauvais Lake in 2007 – the first instance over the initial 3 sampling seasons when more than 2 samples were collected in a single season. This provides a clear example of the need to collect multiple samples over an open-water season in order to understand the true prevalence of MCYST in surface waters.

Climate Change and Prevalence of Toxic Cyanobacteria

The increase in toxin prevalence observed in 2007 is noteworthy (Figure 7) and raises questions as to what principal factors influence cyanobacteria growth and reproduction in Alberta's lakes and reservoirs. Above-normal late-winter/spring climatic conditions and an early spring thaw could advance water column warming. This could cause early onset of water column stabilization and provide better conditions for cyanobacterial growth and surface accumulation. These conditions could allow cyanobacteria to dominate the phytoplankton community in June or July rather than later (Figure 7). Additionally, a protracted open-water season and higher degree of water column warming resulting from early thaw could potentially increase the duration and magnitude of cyanobacterial blooms.

Historical surface water temperature data for lakes collected by AENV reveals only 11 instances out of 3895 recorded measurements (between 1980 through 2007), when water temperatures at 1m depth reached or exceeded 25ºC. Of these, 7 cases were recorded in 2007. The reproductive rate of cyanobacteria is dependent on water temperature. Maximum rate of reproduction for many species increases to an optimum temperature nearing 30ºC. It is likely that above-normal water temperatures occurring in 2007 resulted in increased cyanobacterial abundance and hence greater MCYST prevalence (Figures 6 and 7) and higher peak concentrations (Figure 4; Appendix III, Figure A3).

3.2 Microcystin Analogue Study

In addition to the 172 samples analyzed for total MCYST concentration in 2005, 87 of these were also analyzed for the concentration of specific MCYST analogues including MCLR, MCYR and MCRR via LC-MS/MS. Of the 3 toxin analogues, MCLR was the most prevalent being detected in 60 of the 87 samples (Table 3). The next most prevalent was MCYR and the least was MCRR being detected in 21 and 8 of the 87 samples, respectively. Notably, MCLR was present in all samples containing either MCRR or MCYR with the exception of one.

Information on the occurrence of MCYST analogues (other than MCLR) in Canada's surface waters was limited (prior to this study). For this reason, Health Canada (2002) only considered a maximum acceptable concentration of MCLR when developing its drinking water guideline, citing insufficient data on other MCYST analogues to suggest otherwise. Earlier research suggested MCLR is not the only MCYST produced by cyanobacteria in Alberta's lakes and reservoirs. Bloom material collected from Little Beaver Lake, Alberta in 1991 contained myriad

toxin analogues (in addition to MCLR) including: 1.5 μg/g of MCLL, < 20 ng/g of MCLV, MCLM, MCLF and MCLZ (Z being an unidentified amino acid) and undetermined concentrations of MCLA and MCFR (Boland *et al.*, 1993; Craig *et al.*, 1993). The analogues MCRR and MCLR occurred in a suite of northern Alberta boreal lakes from 1997-98 (Kotak and Zurawell, 2006). Over this period, MCRR was detected in only 3 of 38 phytoplankton samples, while MCLR was detected in 28 of these samples. In two instances however, the concentration of MCRR exceeded that of MCLR (Big Chief Lake: MCRR = 57 μg/g vs. MCLR = 27 μg/g; Cowper Lake: MCRR = 47 μg/g vs. MCLR = 20 μg/g). Similarly, an unidentified MCYST analogue, later determined to be MCLL, was found in concentrations greatly exceeding that of MCLR in phytoplankton collected from Formby Lake, Driedmeat Lake and a northern boreal lake (Kotak and Zurawell, 2006). In contrast, Murphy *et al.* (2003) identified MCRR as the primary analogue in blooms collected from Hamilton Harbour of Lake Ontario, though MCYR and MCLR were also present.

The results from 2005 (Table 3) are consistent with many of these previous findings as MCLR was the most prevalent MCYST analogue in samples collected. The low prevalence of MCRR (occurring in 9% of the samples) is comparable to that of the province's northern boreal lakes (8% occurrence) determined earlier (Kotak and Zurawell, 2006). This is the first study to investigate the occurrence of MCYR in Alberta's surface waters and the results are noteworthy as it was detected in nearly one quarter (24%) of the samples and occurred in the absence of MCLR.

Table 3 Summary of surface water samples with MCYST analogues detected in 2005 by ARCV.

MCYST Analogue	Number of Samples with Detected Analogue	% of Samples with Detected Analogue
MCLR	60	69%
MCYR	21	24%
MCRR	8	9%

A second round of MCYST analogue testing was conducted in 2007 and included MCLF and MCLW in addition to MCLR, MCRR and MCYR. Of the 44 samples tested, 36 (82%) contained MCLR, 6 (14%) contained MCRR, and 2 (5%) contained MCYR (Table 4). No samples contained detectable levels of MCLW or MCLF.

Table 4 Summary of surface water samples with MCYST analogues detected in 2007 by ACFT.

MCYST Analogue	Number of Samples with Detected Analogue	% of Samples with Detected Analogue
MCLR	36	82%
MCYR	2	5%
MCRR	6	14%
MCLF	0	0%
MCLW	0	0%

Microcystin-LR was the most prevalent MCYST analogue in Alberta's surface waters; however, other analogues occur simultaneously and in some cases MCLR may not be the dominant toxin. Occasionally, MCYST analogues may occur in the absence of MCLR. Note that we currently possess very limited capacity to detect and quantify all known toxic analogues, as pure, certified MCYST standards required for analysis by LC-MS/MS or similar instrument-based analytical methods (e.g., HPLC; GC) do not exist in sufficient quantity. Thus, MCYSTs not included in the limited suite of analogues analyzed by ARCV in 2005 (3 analogues) and ACFT in 2007 (5

analogues) could have been present in samples. Comparing results from the analogue analyses conducted by ACFT in 2007 (suite of 5 MCYST analogues) with split-sample results from the PPI assay support this premise. Five of 8 (63%) samples deemed negative or 'non-detected' by the 5-analogue LC-MS/MS scan (12% of the 44 samples overall) did contain a detectable enzyme inhibition response by PPI assay.

It is important to consider that MCYST analogues differ widely in terms of toxicity. Ultimately, MCYSTs cause cell damage and death by binding to, and inhibiting PP1 and PP2A enzymes. These enzymes regulate intracellular signal transduction pathways responsible for a multitude of cell functions including cell division, cell-to-cell signaling and cell metabolism (reviewed in Zurawell *et al.*, 2005). Binding affinity for PP1 and PP2A enzymes varies with MCYST analogue and while influenced by the properties of constituent amino acids, inhibition is largely dependent on the integrity of Adda and D-Glu (Figure 1).

In a comparative PPI assay, researchers at the ACFT demonstrated the difference in the relative ability of MCLR, MCYR, MCRR, MCLF, MCLW, MCLA and MCLY to inhibit PP1. In this study, MCLF was the most potent inhibitor of PP1 with an IC_{50} (concentration of toxin resulting in 50% reduction in PP1 enzyme activity) = 0.12 nMol, followed by MCLR, MCLA, MCLW, MCLY, MCRR and MCYR (Table 5). Mean inhibition - relative to MCLR (Table 5) - demonstrates several toxin analogues to be of similar inhibitory potency for PP1 (i.e., MCLA, MCLW and MCLY - values close to 1) and others to be less so (i.e., MCRR and MCYR - values much greater than 1). It is important to note that relative inhibitory potency of various MCYST congeners to PP2A may differ from that to PP1. Monks *et al.* (2007) showed relative potency to PP1 as MCLF>MCLR>MCLW>MCRR>MCYR and to PP2A as MCLR>MCLW>MCLF>MCYR>MCRR.

Table 5 IC_{50} of PP1 by 7 MCYST analogues and mean inhibition relative to MCLR (ACFT unpublished data). Values for mean inhibition greater than 1 indicate less toxic than MCLR; values less than 1 indicate more toxic than MCLR.

MCYST Analogue	PP1 IC_{50} (nMol)	Mean Inhibition Relative to MCLR
MCLR	0.14	1
MCYR	0.41	2.95
MCRR	0.34	2.43
MCLF	0.12	0.89
MCLW	0.21	1.5
MCLA	0.18	1.25
MCLY	0.23	1.65

Critical to toxicity is the ability of MCYSTs to cross (biological) cell membranes. Though constituent amino acids include both polar (hydrophilic) and non-polar (hydrophobic) residues, all MCYSTs are, albeit to varying degrees, soluble in water (de Maagd *et al.*, 1999). Unlike lipophilic (fat-soluble) substances, the passive uptake of MCYSTs by cells/tissues is limited.

Active uptake of MCYSTs into cells is required for toxicity and is mediated by organic anion transporter polypeptides (OATPs). Several OATPs – OATP1B1 and OATP1B3 – capable of mediating MCYST uptake are specifically expressed in hepatocytes (liver cells) for which bile acid salts among others (e.g., cholate and taurocholate) are the "natural" substrates (Dietrich and Hoeger, 2005; König *et al.*, 2006). Yet another – OATP1A2 – is expressed in a variety of cells including liver cholangiocytes (bile duct epithelial cells) and renal (kidney) and intestinal epithelial cells, but predominantly in blood capillary endothelium of the brain (Fischer *et al.*, 2005; Lee *et al.*, 2005).

The occurrence, type and level of expression (i.e., number of trans-membrane proteins per cell) of OATPs may largely account for the hepato- (liver) toxic nature of MCYSTs. It also corroborates recent evidence indicating the toxins can cross the blood-brain and blood-cerebrospinal fluid barriers and explains observed acute neurotoxicity in those fatally exposed to MCYST in Caruaru, Brazil in 1996 (Dietrich and Hoeger, 2005; Pouria *et al.* 1998).

Here too, differences in constituent amino acids greatly influences the affinity of MCYSTs to OATPs and hence variation in analogue uptake and resulting toxicity. Those containing polar amino acids at variable positions 2 and 4 (Figure 1) are generally less toxic than those with one or more non-polar amino acids. For example, replacement of leucine in position 2 with another non-polar amino acid (e.g., alanine, phenylalanine or tryptophan) maintains toxicity, but substitution with a polar amino acid (e.g., arginine) dramatically reduces it (Stotts *et al.*, 1993).

MCYSTs with polar amino acids in both positions, such as MCRR (arginine, arginine) and MCM(O)R (methionine sulfoxide, arginine), are the least toxic (Zurawell, 2001). Those with only non-polar amino acids in position 2 and 4 are far more toxic. Monks *et al.* (2007) recently demonstrated differences in growth inhibition of OATP1B1 and OATP1B3 transfected cell lines to 5 MCYST analogues. In both instances, the non-polar congeners MCLW and MCLF were more cytotoxic than either MCLR or MCYR, which contain non-polar (leucine or tyrosine, respectively) and polar (arginine) substitutions. MCRR was by far the least cytotoxic (Table 6).

Table 6 Growth inhibition of OATP transfected cells lines by MCYST analogues (adapted from Monks *et al.* 2007).

MCYST Analogue	OATB1B1 IC_{50} (nMol/L)	OATB1B3 IC_{50} (nMol/L)
MCLW	0.3 ± 0.1	0.5 ± 0.4
MCLF	0.4 ± 0.1	0.9 ± 0.9
MCLR	5 ± 51	39 ± 8
MCYR	90 ± 20	45 ± 30
MCRR	3,800 ± 2,300	580 ± 400

Moreover, a genetically modified cell line expressing OATP1B3 seeded on plates incorporating microelectronic sensor arrays allowed real-time monitoring of dynamic responses (i.e., proliferation, apoptosis and morphology change) to MCYSTs by analyzing changes in impedance measurements (Huang *et al.*, Accepted). Comparative toxicity studies based on this real-time cell electronic sensing system (RT-CES) indicate differences in cytotoxicity of 7 MCYST analogues (Table 7). The most hydrophobic of congeners (MCLF, MCLA, MCLY, and MCLW) were more toxic than MCLR, while MCYR and hydrophilic MCRR were less so.

Table 7 RT-CES based cytotoxicity of OATP1B3 cells lines by MCYSTs (ACFT unpublished data).

MCYST Analogue	Mean Cytotoxicity IC_{50} (nMol)
MCLW	0.30
MCLF	0.13
MCLR	1.00
MCYR	2.59
MCRR	7.76
MCLA	0.19
MCLY	0.19

3.3 Anatoxin-a Study

A total of 66 samples were collected in 2005 and analyzed for ATX-a concentration. Of these, 55 were collected from 29 natural lakes and 11 were collected from 6 reservoirs across the province (Appendix II, Table A5). Anatoxin-a was detected in 7 (11%) of the samples collected in 2005 (Figure 8). Four of these contained ATX-a concentrations equal to the limit of detection, and the highest concentration was 0.5 µg/L. During this monitoring program, ATX-a was detected in Hilda, McLeod, Saskatoon, Skeleton (South Basin), Wabamun (West Basin) and Whitefish lakes and in Moonshine Reservoir.

Little data exist on the occurrence of ATX-a in Alberta's surface waters. In part, this may be due to the fact that few laboratories have the capacity to analyze for the toxin. Additionally, compared to MCYSTs, ATX-a is relatively unstable and degrades rapidly making sample collection and preparation difficult. Yet, ATX-a toxicity has been implicated in livestock and wildlife mortalities on several occasions throughout the province. In these historical cases, identification of ATX-a producing cyanobacteria from water samples along with symptoms and gross pathology of affected animals, has led to presumptive conclusions.

The low occurrence and concentrations of ATX-a in samples collected in 2005 is not surprising, as the species of cyanobacteria that commonly produce the toxin rarely dominate the phytoplankton communities of Alberta's bloom-prone lakes. These species comprise only a small percentage of the phytoplankton community. In 2006, a number of lakes and reservoirs experienced blooms of cyanobacteria (primarily *Anabaena* sp.) that could produce ATX-a. However, none of the (10) samples collected in 2006 contained detectable levels of ATX-a.

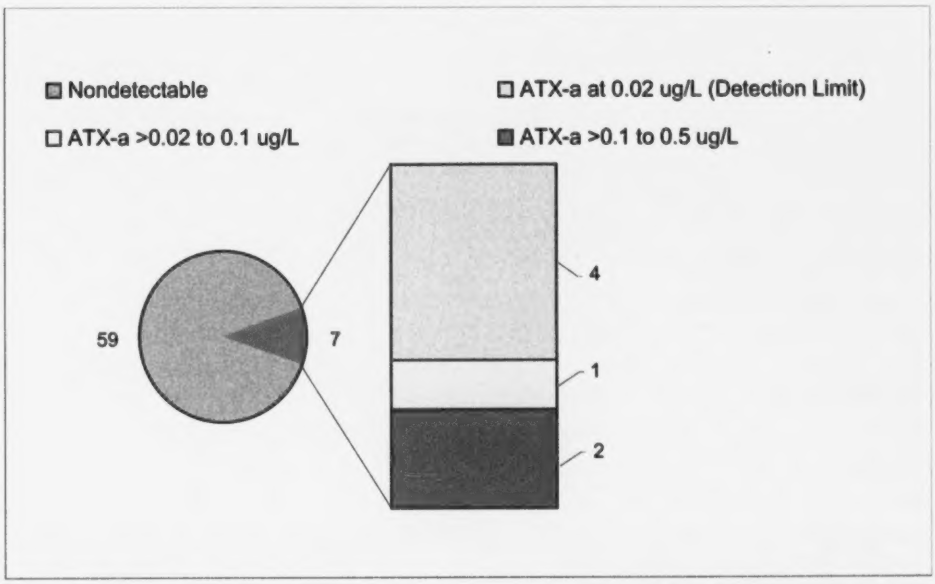

Figure 8 **Number of surface water samples with nondetectable (59 samples; blue portion of pie chart) vs. detectable (7 samples; red portion of pie chart) levels of ATX-a in 2005. The bar summarizes the number of samples with ATX-a at various incremental concentrations.**

3.4 β-*N*-Methylamino-L-Alanine (BMAA) Study

Unlike the other studies presented above, a large component of the BMAA study in 2005 was devoted to developing methods for the isolation, identification and quantification of the compound from surface water samples (this constitutes Phase 1 of BMAA research). The first consideration was to confirm that a standard (reference) solution of BMAA could be detected by standard amino acid analysis (AAA) and LC-MS/MS. A standard solution of BMAA was detected by AAA at a detection limit of 0.02 μMol. The standard could also be detected and molecular weight determined using matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI ToF-MS).

The next step was to analyze water samples collected from lakes with obvious cyanobacteria populations. In the first attempt (Prep A), a 100-ml water sample was lyophilized (freeze-dried) yielding about 10 mg of solid material. Results of AAA of Prep A indicated the total amino acid content was low and BMAA was not detectable. Next (Prep B), a 1 L water sample was filtered through cellulose fiber filter paper (Whatman #1, 11-μm pore size) yielding about 8 mg of solid material. Total amino acid content of Prep B was also low and BMAA could not be detected.

At this point we acknowledged the need to further concentrate fresh water samples prior to analyses. Sample preparation methodology was altered to include filtering known volumes of freshly-collected water samples through 1.6-μm pore size glass fiber filters (Whatman GF/A) in the same fashion as preparing samples for the determination of chlorophyll-*a*. Four samples (one each from Baptiste Lake South Basin, Sturgeon Lake, Winagami Lake and Moonshine Reservoir) were analyzed following this procedure. Results of AAA indicated that a peak (compound) could be detected in these concentrated samples at a retention time consistent with that of pure BMAA standard (about 47 min elution time). Co-injection of a BMAA standard with sample was taken as further evidence the peak in the chromatogram was BMAA. Since these samples were not subjected to mass spectrometry analysis, the identity of the compound (peak) was not confirmed unequivocally and the presence of BMAA remains presumptive at this point. Concentrations of presumptive BMAA were determined by correlating peak area with that of a set of serially diluted BMAA standards. The concentrations of presumptive BMAA in the 4 samples were 41, 27, 23 and 12 μg per injection volume for Baptiste Lake South Basin, Sturgeon Lake, Winagami Lake and Moonshine Reservoir, respectively, equaling BMAA concentrations of 12, 8, 7 and 4 μg/sample or 48, 32, 28 and 16 μg/L lake water.

Without exception, cyanobacteria were the most abundant of the phytoplankton groups in all 4 samples based on percent abundance (Table 8). In terms of absolute abundance (i.e., number of cells per L), Baptiste Lake South Basin had the greatest density of cyanobacteria. Its community was dominated by the cyanobacteria, *Aphanizomenon flos-aquae* and *Coelosphaerium naegelianum*. Sturgeon Lake contained the 2nd highest density of cyanobacteria, which comprised greater than 90% of the overall phytoplankton community. The dominant cyanobacterium in Sturgeon Lake was *Aphanizomenon flos-aquae*, but *Oscillatoria sp.*, *Chroococcus dispersus* and an unidentified cyanophyte were abundant. Cyanobacteria density within Winagami Lake and Moonshine Reservoir was lower than in either Baptiste or Sturgeon lakes (Table 8), however species diversity was greater. *Coelosphaerium naegelianum* dominated the phytoplankton community within Moonshine Reservoir, but *Aphanizomenon flos-aquae*, *Anabaena circinalis*, *Chroococcus dispersus* and an unidentified species were abundant as well. Winagami Lake contained the most diverse assemblage of cyanobacteria including *Aphanizomenon flos-aquae*, *Anabaena flos-aquae*, *Microcystis incerta*, *Oscillatoria sp.*, *Aphanocapsa delicatissima*, *Chroococcus dispersus*, and 3 additional unidentified cyanophytes.

Table 8 **Summary of cyanobacteria abundance, relative abundance and biomass in water samples analyzed for BMAA in 2005.**

Location	Abundance of Cyanobacteria (# cells/L)	% Relative Abundance of Cyanobacteria	Biomass of Cyanobacteria (ug/L)
Winagami Lake	5,885,942	79%	20619
Moonshine Lake Reservoir	4,183,155	72%	753
Sturgeon Lake	13,517,320	90%	11744
Baptiste Lake South Basin	19,422,960	75%	16590

Though Baptiste and Sturgeon lakes had the greatest abundance of cyanobacteria of the 4 samples, Winagami Lake had the greatest biomass of cyanobacteria per L of sample (Table 8). With the exception of Moonshine Reservoir (15.4%), cyanobacteria comprised greater than 50% of the relative biomass in all of the samples (Figure 9). This was most evident in Baptiste Lake where 96.4% of the biomass was attributed to cyanobacteria.

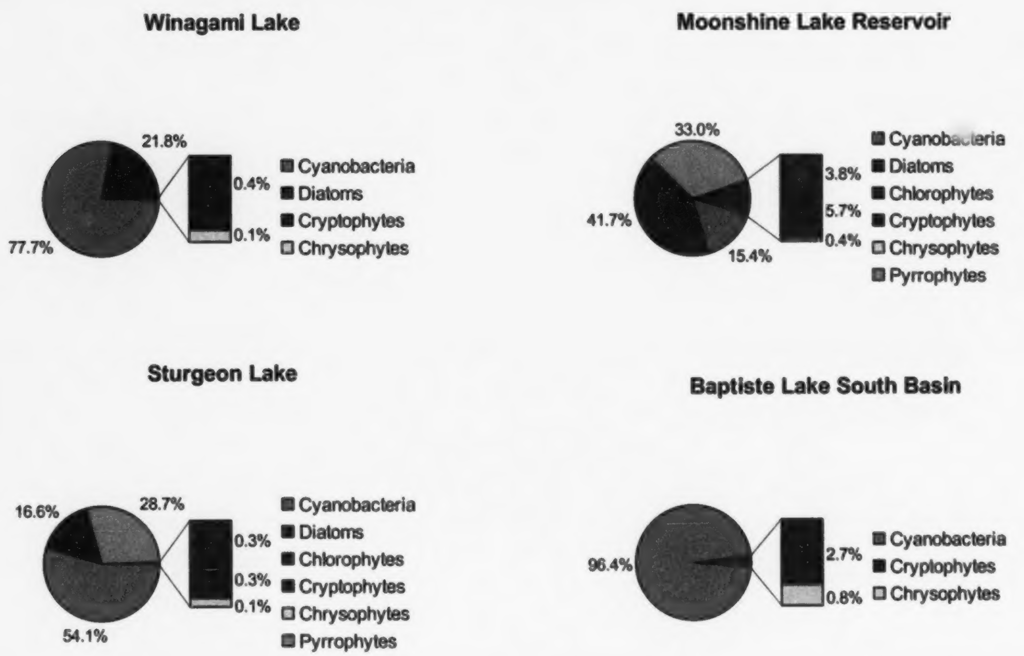

Figure 9 **Pie charts depicting the relative biomass of phytoplankton groups within samples collected for BMAA analysis from Winagami Lake, Moonshine Lake Reservoir, Sturgeon Lake and Baptiste Lake South Basin. The bar presents the data for phytoplankton groups comprising less than 10% or the overall biomass.**

The overwhelming prevalence of cyanobacteria has implications for the occurrence and concentration of BMAA in these lake water samples. Though data are limited, it appears the ambient concentration of BMAA may be highly correlated with the abundance and biomass of cyanobacteria. If future studies confirm the identity of the compound presumed to be BMAA, these findings will support recent studies by Cox *et al.* (2005) that suggested most species of cyanobacteria (90% of the species studied) produce BMAA.

Method development for the analysis of BMAA continued in 2007 in collaboration with the ACFT. An LC-MS/MS method to identify and quantify a fluorescent derivative of BMAA following reverse-phase separation on a C_{18} column was established (see Appendix I -

Laboratory Methods). BMAA was successfully identified and quantified in hydrolyzed cycad leaf by this method, which served as a positive biological reference material. Hydrolyzed cyanobacteria and fish filet tissue samples were spiked with BMAA (5 - 1000 μg/g) to validate the method. Method recovery was 85 - 115% with a relative standard deviation of 6 - 10%.

In addition, ACFT compared results of derivatized BMAA with a modified LC-MS/MS method employing Hydrophilic Interaction LC (HILIC) normal-phase column without derivatization (Kubo *et al.*, 2008; Rosén and Hellenäs, 2008). Kubo *et al.* (2008) argue HILIC is more effective than traditional reverse-phase (C_{18}) columns for separating BMAA from other closely related amino acids due to an increased efficiency that results from the rapid evaporation of organic solvent during electrospray ionization. The 2 methods were in agreement. With these new methods established, ACFT is continuing testing of archived cyanobacteria samples in 2009 to confirm if the putative toxin exists in our surface waters. Testing of fish samples will also be conducted in the future. Data will be presented at a later time.

4.0 GENERAL DISCUSSION

Cyanotoxin monitoring was incorporated into Alberta's Lake and Reservoir Monitoring Program in 2005 with the goal of determining the prevalence of MCYST in Alberta. In addition, funding was obtained to determine the occurrence and concentrations of ATX-a and several MCYST analogues in many of these lakes and reservoirs. MCYST was very prevalent over the period of study (2005 - 2008), occurring on at least one sampling occasion in 75% (2005) to 96% (2007) of lakes and reservoirs in a given year. In terms of samples overall, toxin was detected in 48% (2005) to 76% (2007) of those collected in any given open-water period.

High prevalence of MCYST noted in 2007 is of concern and indicates that all Alberta lakes and reservoirs occasionally experience some degree of toxin-producing cyanobacteria regardless of trophic status. Climate could be an important factor in determining the abundance of cyanobacteria in our lakes and reservoirs. Warm surface water temperatures in 2007 likely contributed to higher cyanobacterial growth in all surface waters regardless of nutrient conditions.

The period of onset and severity of cyanobacterial blooms may be, in part, determined by water temperature, as they occurred earlier (early July as opposed to August) and were more extensive in area and duration in many productive (eutrophic and hypereutrophic) systems than usual. Increasing air temperatures could result in earlier ice-off and lengthier ice-free seasons. Protracted periods of water column stability combined with warmer overall water temperatures would undoubtedly favor growth and reproduction of cyanobacteria and likely result in greater frequency, duration and severity of potentially toxic blooms.

Though variable year to year, whole-lake concentrations of MCYST appear to be low (generally < 1.5 µg/L total MCYST). The highest concentrations were recorded in 2007; in that year, a greater proportion of samples overall, contained both detectable MCYST and elevated toxin levels (> 1.5 µg/L total MCYST) than in any other year studied. And in two instances, concentrations exceeded 20 µg/L total MCYST – the newly proposed recreational guideline value for Canada. Here again, factors controlling cyanobacterial growth, reproduction, community composition and toxin production, such as water temperature and nutrient availability, may principally influence MCYST concentrations.

It is important to note these concentrations are based on depth integrated water samples collected from the surface to the bottom of the euphotic zone. While this provides an indication of the average whole-lake MCYST concentrations, it does not adequately assess peak concentrations that may occur in areas of densely accumulated cyanobacteria. Surface-grab samples (i.e., uppermost 10 cm of a water body) of severe blooms in embayment and near shoreline areas often yield much higher toxin levels. Notably, concentrations as high as 15,000 µg/L total MCYST – four to five orders of magnitude higher than typical whole-lake concentrations – have been documented in concentrated blooms samples from hypereutrophic recreational lakes. To determine risk of toxic blooms to recreational users, frequent (weekly) surface sampling of near-shore areas including public beaches and swimming areas would be more appropriate, as is conducted by Alberta Health Services.

It is evident that MCYSTs do occur in less productive waters corroborating evidence that many of Alberta's oligo- and mesotrophic lakes and reservoirs experience periodic blooms of metalimnetic cyanobacteria (e.g., *Planktothrix* sp.). In the one instance with sufficient evidence of a metalimnetic bloom, a discrete sample taken at depth contained MCYST. Metalimnetic

blooms are sporadic in nature – they may be brief and yet re-occurring events in a given water body throughout the open water season. This implies site (lake/reservoir) selection based solely on nutrient status or chlorophyll-*a* concentration (i.e., oligo-, meso-, eu- and hypereutrophic) may be inappropriate when designing monitoring programs to assess the risk of MCYST exposure, as toxin occurs in all lentic (i.e., still or slow moving waters) systems regardless of trophic classification. Timing and frequency of monitoring is also an important consideration in terms of being able to observe the dynamic nature of toxin-producing cyanobacteria and detect their toxins. A single sample collected over a season is insufficient to draw conclusions or evaluate risk regarding the prevalence of cyanotoxin in Alberta's lakes and reservoirs. Other factors including consumptive use by humans (e.g., raw source for drinking water) and livestock (watering) and degree of direct (public beaches for swimming) and indirect (boating and fishing) recreational exposure need to be considered.

The prevalence of MCYST in oligo- and mesotrophic lakes and reservoirs suggests all drinking water treatment facilities drawing water from lentic (relatively still waters: lakes, ponds, reservoirs and irrigation canals) systems – regardless of trophic status – may risk toxin exposure. This includes utilities withdrawing and impounding water from lotic systems (i.e., fast-flowing streams and rivers) within reservoirs; conditions in storage reservoirs can be conducive for cyanobacteria growth. This also implies that no untreated water from these sources should be directly consumed or used for cooking (MCYSTs are heat stable and are not destroyed with boiling) or bathing (inhalation of water mist or spray is a recognized route of MCYST exposure).

Results from the MCYST analogue studies support the premise that MCLR is a common toxin in Alberta's surface waters. It is apparent that other analogues, such as MCRR and MCYR, may also be prevalent and can occur in the absence of MCLR or at concentrations exceeding that of MCLR. Recognizing we have a limited capacity to measure all of the known toxic congeners, it is not unrealistic to presume others occur in our lakes and reservoirs as well.

The various toxin analogues differ in terms of toxicity as a result of inherent properties of individual constituent amino acids. Unequal inhibition of PP1 and PP2A by various MCYSTs only partly account for the disparities in overall toxicity. In addition, the active uptake of toxin by MCYST specific OATPs is required for toxicity. Binding and uptake rates of MCYSTs vary with analogue and specific OATP with the most hydrophobic of congeners (MCLF, MCLA, MCLY, and MCLW) being generally more toxic than MCLR and hydrophilic forms (MCYR and MCRR). These results call into question the current drinking water guideline based solely on the concentration of MCLR. It is evident that further consideration be given to other, potentially more toxic, MCYST analogues if a guideline is to be protective against this group of toxins. A guideline that considers cumulative toxicity of MCYSTs as representing the potential threat to human health via drinking water – perhaps based on total MCLR toxicity equivalents (as adopted in Australia) – appears more appropriate for use in Alberta.

The neurotoxin, ATX-a, occurred infrequently and at low concentrations in Alberta's lakes and reservoirs monitored in 2005. During the summer of 2006, blooms were uncharacteristically dominated by potentially neurotoxic species (*Anabaena* sp.) and several water samples were collected for ATX-a analysis. However, none of these samples contained ATX-a at or above the analytical limit of detection. It appears the risk posed by ATX-a is much lower than that of hepatotoxic MCYSTs. Testing for ATX-a need only occur if circumstances dictate, such as wildlife or livestock mortality.

Research into the detection and quantification of BMAA was initiated in 2005. The majority of effort during the 4 year period was devoted to developing methods of sample collection,

preparation and analysis. Analysis of several samples indicates that BMAA may occur at detectable concentrations in Alberta's lakes and reservoirs. Though data are limited, it appears that the ambient concentration of presumptive BMAA may be highly correlated with the abundance and biomass of cyanobacteria. Given that some research indicates most cyanobacteria can produce BMAA; this putative toxin may be more prevalent than other cyanotoxins in Alberta. Research on BMAA is continuing with support of the ACFT. If BMAA is confirmed in our lakes and research shows it to be a causative agent of ALS/PDC or other neurodegenerative diseases, the risk to human health will need to be assessed.

5.0 CONCLUSIONS

- Microcystins occur in a high percentage of Alberta lakes and reservoirs. Several analogues are present.
- Microcystins are found in oligo- and mesotrophic lakes, indicating that not only eutrophic/hypertrophic lakes with visible blooms contain toxins.
- Average concentrations can periodically exceed 20 μg/L of total microcystin - the draft RWQ guideline value for surface waters. It is recognized that toxin concentrations in near-shore bloom accumulations can be significantly higher than levels in open-water.
- Climate may influence MCYST prevalence and concentrations.
- Anatoxin-*a* is infrequent and at low concentrations and appears to pose less risk than MCYST.
- BMAA analytical methods are under development. Risk to human health needs further assessment.

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Appendix I Laboratory Methods

Total Microcystin Analysis

Immediately following collection, aliquots of euphotic integrated composite water samples were poured into pre-rinsed 20mL plastic scintillation vials, cooled, and then stored at -20ºC until analyzed. Total MCYST concentrations were determined with a colorimetric protein phosphatase inhibition (PPI) assay as specified by An and Carmichael (1994). Analyses were conducted by ARC Vegreville.

The PPI assay was chosen for this study over instrument-based analytical methods, such as high performance liquid chromatography (HPLC), fast atom bombardment mass spectrometry (FABMS) and GC-MS, because of its sensitivity to detect trace MCYST concentrations and its ability to estimate 'total' MCYST concentration by quantifying an overall inhibitory response of the protein phosphatase Type 1c enzyme by bioactive MCYSTs. The PPI assay is based on the actual mode of MCYST toxicity, that is, the irreversible binding and subsequent inhibition of protein phosphatase type 1c and 2A (PP1 and PP2A). It quantifies all unbound (i.e., those not bound to endogenous PP1 or PP2A), bioactive (i.e., MCYSTs capable of inhibiting PP1) toxin analogues, though differentiation between and identification of various congeners is not possible (Zurawell, 2002).

Total MCYST concentrations in composite water samples were extrapolated from curves plotting PP1 inhibition by MCLR standards and are thus expressed as mass (μg) of MCLR equivalents/L. The assay detection limit (i.e., lowest measureable level of MCYST inferred from the standards dose-inhibition response curve) is approximately 0.07 μg MCLR eq./L. However, test plate conditions (e.g., PP1c enzyme activity and assay buffer concentrations and pH) can influence the inhibition response of the assay and lower limits can range from 0.05 to 0.09 μg MCLR eq./L. Considering this, the lower limit of detection for all samples analyzed by PPI assay was set at 0.1 μg MCLR eq./L and lesser concentrations were regarded as 'non-detected'.

It is recognized that other natural compounds of cyanobacterial (i.e., nodularin and motuporin) and non-cyanobacterial origin (i.e., okadaic acid, calyculin-A and tautomycin) also inhibit PP1; it was assumed they were not present in our samples for the following reasons. Nodularins occur in brackish and marine species of cyanobacteria including *Nodularia spumigena* typically occurring in the Baltic Sea and brackish water estuaries and coastal lakes. Similarly, motuporin (also known as nodularin-V) originates from cyanobacterial symbionts associated with the tropical sponge *Theonella swinhoei*. Okadaic acid, a causative agent of diarrhetic shellfish poisoning, is a polyether fatty acid (polyketide) originating from unicellular, marine dinoflagellates primarily of the genus *Prorocentrum*. Calyculin-A is a phosphorylated polyketide also isolated from a marine sponge, *Discodermia calyx*. Thus, the presence of these PP1 inhibitors is highly unlikely. In contrast, tautomycin, a polyketide structurally related to okadaic acid, is produced by the soil bacterium *Streptomyces spiroverticillatus* (MacKintosh and Klumpp, 1990). Theoretically, soil bacteria within the watershed could be a source of this PP1 inhibitor. However, as *Streptomyces* is closely associated with rooted terrestrial vegetation, it is unlikely that tautomycin occurs in sufficient concentrations to influence PP inhibition in surface water samples.

Microcystin Analogue Analysis

Microcystin analogues (MCLR, MCYR and MCRR in 2005 by ARC Vegreville; MCLR, MCLF, MCLW, MCYR and MCRR in 2007 by ACFT) were separated and quantified by liquid

chromatography linked tandem mass spectrometry (LC-MS/MS). Aliquots of euphotic integrated composite water samples were poured into pre-rinsed 500mL plastic bottles immediately following collection and stored at -20°C until analyzed. Following thawing in the laboratory, samples were sonicated to disrupt cyanobacterial cell membranes and liberate toxin.

2005 Samples

Sonicated samples were passed through Waters Oasis® HLB (Hydrophilic-Lipophilic Balance reverse-phase sorbent) solid-phase extraction (SPE) cartridge. MCYSTs were eluted with 6 mL methanol then evaporated to a final 1-mL volume. Samples were separated on reverse-phase C_{18} column linked to MS with electrospray ionization (ESI). Multiple reactions monitoring (MRM) transition was: MCRR 520 - 135; MCLR 995 - 135; and MCYR 1045 - 135.

2007 Samples

Sonicated samples were filtered through PES (polyethersulfone) syringe-type filter cartridge prior to direct injection on an Agilent 1100 LC without further sample preparation. An 80 µL sample volume was injected on a BDS Hypersil reverse-phase C_{18} column (100 mm x 2.1mm, 5µ particle size) and MCYSTs eluted with a gradient of 0.1% formic acid in de-ionized water and 0.1% formic acid in acetonitrile (mobile phase). The column was at 40°C with a flow rate of 0.3 mL/min. The tandem MS analyses were conducted with a Sciex API 4000 Triple Quadrupole Mass Spectrometer operated in ESI positive mode. MCYST identification and quantification was performed based on the analogue's retention time and two MRM transition (in standard and unknown sample).

Anatoxin-a Analysis

Aliquots of euphotic integrated composite water samples were poured into pre-rinsed 500mL plastic bottles and stored at -20°C until analyzed by ARC Vegreville. Following thawing in the laboratory, samples were sonicated to disrupt cyanobacterial cell membranes and liberate toxin. Samples were passed through Waters Oasis® HLB SPE cartridge and eluted with 6mL methanol. These were evaporated to dryness and derivatized in TFAA (trifluoroacetic anhydride) with heating to 95°C for 1 hour. Samples were evaporated to remove excess TFAA and reconstituted in 100 µL of ethyl acetate. ATX-a was separated and quantified by GC-MS ion trap.

β-*N*-Methylamino-L-Alanine (BMAA) Methods

Researchers at ACFT developed an LC-MS/MS-based method for determining BMAA. Reference standards of BMAA and hydrolyzed cycad leaf, cyanobacteria and fish samples were derivatized, pre-column, using Waters' AccQ-Fluor™ reagent. Derivatized BMAA and an internal standard of α-aminobutyric acid were separated with an Agilent 1100 Liquid Chromatograph fitted with a Hypersil BDS reverse-phase C18 column (100 mm x 2.1mm i.d., 5-µm particle size) under gradient conditions maintained at 40°C. BMAA and α-aminobutyric acid were identified and quantified using a Sciex API 4000 Triple Quadrupole Mass Spectrometer in MRM mode. MRM transitions were: 459/171 and 459/289 for BMAA and 274/171 for α-aminobutyric acid. Identification and quantification was performed based on the two MRM transitions combined with the retention time. The detection limit for total BMAA was 2 µg/g dry

weight (2 pg on column). Final BMAA concentrations were calculated based on known sample volume and expressed as µg/L of lake water.

In addition, BMAA was analyzed with a modified LC-MS/MS method employing Hydrophilic Interaction LC (HILIC) normal-phase column without derivatization (Kubo *et al.*, 2008; Rosén and Hellenäs, 2008). The LC column was a TSK-gel Amide 80 HILIC column (150 mm x 2.0 mm i.d., 5-µm particle size, Tosoh Bioscience Gmbh., Japan). BMAA was separated and eluted from column in (A) 0.05% aqueous TFA and a 90-60% linear gradient of (B) acetonitrile (mobile phase) over 15 min; flow rate was 0.3 mL/min. at 40°C. MRM transitions were: 119.1/102, 119.1/44 and 119.1/88. The detection limit for total BMAA was 1 µg/g dry weight (1 pg on column).

Phytoplankton Community Analysis

Aliquots of composite water were poured in 100-mL amber bottles and preserved in Lugol's solution. These were submitted to Dr. C. Earle (Edmonton, Alberta) for phytoplankton community analysis (identification and enumeration). Analyses were performed on subsamples following acclimation to room temperature conditions, adequate re-suspension and settling by the Utermöhl method. Phytoplankton was identified to the lowest confirmed level of classification. Enumeration involved counting transects (strips) along the length of the chamber at several magnifications.

Appendix II List of Lakes/Reservoirs

Table A1 List of lakes and reservoirs sampled for total microcystin in 2005 including trophic status, parent monitoring program and source of funding for toxin analyses.

Site Name	Trophic Status	Program	Funding
Baptiste Lake North Basin	Hypereutrophic	LTLN	LTLN
Baptiste Lake South Basin	Hypereutrophic	LTLN	LTLN
Beauvais Lake	Mesotrophic	Prov. Parks	Prov. Parks
Bluet Lake (Garnier Lake South)	Eutrophic	ALMS	WRUG
Crimson Lake	Mesotrophic	Prov. Parks	Prov. Parks
Dillberry Lake	Mesotrophic	Prov. Parks	WRUG
Elkwater Lake	Mesotrophic	Prov. Parks	WRUG
Fishing Lake	Hypereutrophic	ALMS	WRUG
Fork Lake	Eutrophic	ALMS	WRUG
Frog Lake	Mesotrophic	ALMS	WRUG
Garnier Lake North	Mesotrophic	ALMS	WRUG
Goose Lake	Hypereutrophic	ALMS	WRUG
Gregg Lake	Oligotrophic	Prov. Parks	WRUG
Gregoire Lake	Eutrophic	Prov. Parks	WRUG
Hilda Lake	Mesotrophic	ALMS	WRUG
Island Lake	Eutrophic	ALMS	WRUG
Jarvis Lake	Oligotrophic	Prov. Parks	WRUG
Kananaskis Lake Lower	Oligotrophic	Prov. Parks	WRUG
Kananaskis Lake Upper	Oligotrophic	Prov. Parks	WRUG
Kehiwin Lake	Hypereutrophic	ALMS	WRUG
Long Lake (Near Boyle)	Eutrophic	Prov. Parks	WRUG
McLeod Lake (East)	Eutrophic	Prov. Parks	Prov. Parks
Miquelon Lake	Mesotrophic	Prov. Parks	WRUG
Moonshine Lake Reservoir	Eutrophic	Prov. Parks	Prov. Parks
Moore (Crane) Lake	Eutrophic	ALMS	WRUG
Moose Lake	Eutrophic	ALMS	WRUG
Newell Lake Reservoir	Mesotrophic	Prov. Parks	Prov. Parks
Pine Lake	Eutrophic	ALMS	WRUG
Reesor Lake Reservoir	Eutrophic	Prov. Parks	Prov. Parks
Saskatoon Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Skeleton Lake North Basin	Eutrophic	ALMS	WRUG
Skeleton Lake South Basin	Eutrophic	ALMS	WRUG
Spruce Coulee Reservoir	Mesotrophic	Prov. Parks	Prov. Parks
Steele (Cross) Lake	Hypereutrophic	Prov. Parks	WRUG
Sturgeon Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Wabamun Lake East Basin	Eutrophic	LTLN	LTLN
Wabamun Lake West Basin	Eutrophic	LTLN	LTLN
Whitefish Lake	Mesotrophic	ALMS	WRUG Funded
Winagami Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Wolf Lake	Mesotrophic	ALMS	WRUG

Trophic status based on mean summer chlorophyll-*a* concentration. ALMS = Alberta Lake Management Society's Lakewatch Program; LTLN = Long-term Lake Network Program; WRUG = Water Research User's Group funded project.

Table A2 List of lakes and reservoirs sampled for total microcystin in 2006 including trophic status, parent monitoring program and source of funding for toxin analyses.

Site Name	Trophic Status	Program	Funding
Baptiste Lake North Basin	Hypereutrophic	BISL	Cyanotoxin Program
Baptiste Lake South Basin	Hypereutrophic	BISL	Cyanotoxin Program
Bear Trap Lake	Mesotrophic	ALMS	Cyanotoxin Program
Beauvais Lake	Eutrophic	Prov. Parks	Cyanotoxin Program
Big Lake	Eutrophic	ALMS	Cyanotoxin Program
Buck Lake	Eutrophic	Central AB Lakes	Central AB Lakes
Clear (Barnes) Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Cooking Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Crimson Lake	Mesotrophic	Prov. Parks	Cyanotoxin Program
Dillberry Lake	Mesotrophic	Prov. Parks	Prov. Parks
Elkwater Lake	Mesotrophic	Prov. Parks	Prov. Parks
Ethel Lake	Mesotrophic	LTLN	LTLN
Fishing Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Frog Lake	Mesotrophic	ALMS	Cyanotoxin Program
Gleniffer Lake (Dickson Dam Res.)	Oligotrophic	Central AB Lakes	Central AB Lakes
Gregg Lake	Oligotrophic	Prov. Parks	Prov. Parks
Gregoire Lake	Eutrophic	Prov. Parks	Prov. Parks
Gull Lake AENV	Eutrophic	Central AB Lakes	Central AB Lakes
Gull Lake ALMS	Eutrophic	ALMS	Cyanotoxin Program
Hilda Lake	Mesotrophic	ALMS	Cyanotoxin Program
Island Lake	Eutrophic	BISL	Cyanotoxin Program
Jarvis Lake	Oligotrophic	Prov. Parks	Prov. Parks
Kananaskis Lake Lower	Oligotrophic	Prov. Parks	Prov. Parks
Kananaskis Lake Upper	Oligotrophic	Prov. Parks	Prov. Parks
Lac Sante	Eutrophic	ALMS	Cyanotoxin Program
Long Lake (Near Boyle)	Eutrophic	Prov. Parks	Prov. Parks
McLeod Lake (East)	Eutrophic	Prov. Parks	Cyanotoxin Program
Miquelon Lake	Mesotrophic	Prov. Parks	Prov. Parks
Mons Lake	Eutrophic	ALMS	Cyanotoxin Program
Moonshine Lake Reservoir	Eutrophic	Prov. Parks	Cyanotoxin Program
Moore (Crane) Lake	Eutrophic	ALMS	Cyanotoxin Program
Moose Lake	Eutrophic	ALMS	Cyanotoxin Program
Muriel Lake	Mesotrophic	ALMS	Cyanotoxin Program
Nakamun Lake	Hypereutrophic	LTLN	LTLN
Newell Lake Reservoir	Mesotrophic	Prov. Parks	Cyanotoxin Program
Pigeon Lake	Eutrophic	Central AB Lakes	Central AB Lakes
Pine Lake	Eutrophic	ALMS	Cyanotoxin Program
Red Deer Lake	Hypereutrophic	Central AB Lakes	Central AB Lakes
Reesor Lake Reservoir	Eutrophic	Prov. Parks	Cyanotoxin Program
Sandy Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Saskatoon Lake	Hypereutrophic	Prov. Parks	Cyanotoxin Program
Skeleton Lake South Basin	Eutrophic	ALMS	Cyanotoxin Program
Spruce Coulee Reservoir	Mesotrophic	Prov. Parks	Cyanotoxin Program
Steele (Cross) Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Sturgeon Lake	Hypereutrophic	Prov. Parks	Cyanotoxin Program
Sylvan Lake	Mesotrophic	ALMS	Cyanotoxin Program
Tucker Lake	Eutrophic	ALMS	Cyanotoxin Program
Winagami Lake	Hypereutrophic	Prov. Parks	Cyanotoxin Program
Wizard Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Wolf Lake	Mesotrophic	ALMS	Cyanotoxin Program

Trophic status based on mean summer chlorophyll-a concentration. ALMS = Alberta Lake Management Society's Lakewatch Program; BISL = Baptiste, Island and Skeleton Lakes Stewardship Society; LTLN = Long-term Lake Network Program.

Table A3 List of lakes and reservoirs sampled for total microcystin in 2007 including trophic status, parent monitoring program and source of funding for toxin analyses.

Site Name	Trophic Status	Program	Funding
Baptiste Lake North Basin	Hypereutrophic	LTLN	LTLN
Baptiste Lake South Basin	Hypereutrophic	LTLN	LTLN
Bear Trap Lake	Mesotrophic	ALMS	Cyanotoxin Program
Beauvais Lake	Mesotrophic	Prov. Parks	Prov. Parks
Lac Bellevue	Mesotrophic	ALMS	Cyanotoxin Program
Bittern Lake	Eutrophic	Central AB Lakes	Central AB Lakes
Clairmont Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Clear Lake	Eutrophic	Southern L&R	Cyanotoxin Program
Clear (Barnes) Lake	Oligotrophic	ALMS	Cyanotoxin Program
Cooking Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Crimson Lake	Mesotrophic	Prov. Parks	Prov. Parks
Dillberry Lake	Mesotrophic	Prov. Parks	Prov. Parks
Driedmeat Lake	Hypereutrophic	Central AB Lakes	Central AB Lakes
Eagle Lake	Eutrophic	Southern L&R	Cyanotoxin Program
Elkwater Lake	Mesotrophic	Prov. Parks	Prov. Parks
George Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Gregg Lake	Oligotrophic	Prov. Parks	Prov. Parks
Gregoire Lake	Eutrophic	Prov. Parks	Prov. Parks
Hilda Lake	Mesotrophic	ALMS	Cyanotoxin Program
Jackfish Lake (Near Carvel)	Eutrophic	Central AB Lakes	Central AB Lakes
Jarvis Lake	Oligotrophic	Prov. Parks	Prov. Parks
Kananaskis Lake Upper	Oligotrophic	Prov. Parks	Prov. Parks
Kehiwin Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Laurier Lake	Mesotrophic	ALMS	Cyanotoxin Program
Long Island North Basin	Mesotrophic	ALMS	Cyanotoxin Program
Long Island South Basin	Mesotrophic	ALMS	Cyanotoxin Program
Long Lake (Near Boyle)	Eutrophic	Prov. Parks	Prov. Parks
Marie Lake	Mesotrophic	ALMS	Cyanotoxin Program
Matchayaw Lake	Hypereutrophic	ALMS	Cyanotoxin Program
McLeod Lake (East)	Mesotrophic	Prov. Parks	Prov. Parks
Miquelon Lake	Mesotrophic	Prov. Parks	Prov. Parks
Moonshine Lake Reservoir	Hypereutrophic	Prov. Parks	Prov. Parks
Moore (Crane) Lake	Mesotrophic	ALMS	Cyanotoxin Program
Newell Lake Reservoir	Oligotrophic	Prov. Parks	Prov. Parks
Pine Coulee Res. North Basin	Eutrophic	Southern L&R	Cyanotoxin Program
Pine Coulee Res. South Basin	Mesotrophic	Southern L&R	Cyanotoxin Program
Pine Lake	Hypereutrophic	Southern L&R	Cyanotoxin Program
Reesor Lake Reservoir	Hypereutrophic	Prov. Parks	Prov. Parks
Lac Sante	Mesotrophic	ALMS	Cyanotoxin Program
Saskatoon Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Shorncliffe Lake	Eutrophic	Central AB Lakes	Central AB Lakes
Siler (Stoney) Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Spruce Coulee Reservoir	Mesotrophic	Prov. Parks	Prov. Parks
Steele (Cross) Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Sturgeon Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Thunder Lake	Hypereutrophic	Central AB Lakes	Central AB Lakes
Tucker Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Twin Valley Reservoir	Eutrophic	Southern L&R	Cyanotoxin Program
Wapasu Lake	Mesotrophic	ALMS	Cyanotoxin Program
Winagami Lake	Eutrophic	Prov. Parks	Prov. Parks
Wolf Lake	Mesotrophic	ALMS	Cyanotoxin Program

Trophic status based on mean summer chlorophyll-a concentration. ALMS = Alberta Lake Management Society's Lakewatch Program; LTLN = Long-term Lake Network Program; Southern L&R = Southern Region Lakes and Reservoirs.

Table A4 List of lakes and reservoirs sampled for total microcystin in 2008 including trophic status, parent monitoring program and source of funding for toxin analyses.

Site Name	Trophic Status	Program	Funding
Adamson Lake	Hypereutrophic	Elk Island Park	Elk Island Park
Amisk Lake	Mesotrophic	ALMS	Cyanotoxin Program
Astotin Lake	Hypereutrophic	Elk Island Park	Elk Island Park
Bear Trap Lake	Mesotrophic	ALMS	Cyanotoxin Program
Beauvais Lake	Mesotrophic	Prov. Parks	Prov. Parks
Beaver Lake	Mesotrophic	ALMS	Cyanotoxin Program
Blackfalds Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Clear Lake	Mesotrophic	Southern L&R	Cyanotoxin Program
Clear (Barnes) Lake	Mesotrophic	ALMS	Cyanotoxin Program
Cow Lake	Oligotrophic	Central AB Lakes	Central AB Lakes
Crimson Lake	Mesotrophic	Prov. Parks	Prov. Parks
Dillberry Lake	Mesotrophic	Prov. Parks	Prov. Parks
Elkwater Lake	Mesotrophic	Prov. Parks	Prov. Parks
Ethel Lake	Mesotrophic	LTLN	LTLN
Goose Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Gregg Lake	Oligotrophic	Prov. Parks	Prov. Parks
Gregoire Lake	Eutrophic	Prov. Parks	Prov. Parks
Gull Lake	Eutrophic	Central AB Lakes	Central AB Lakes
Hastings Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Jarvis Lake	Oligotrophic	Prov. Parks	Prov. Parks
Kananaskis Lake Lower	Oligotrophic	Prov. Parks	Prov. Parks
Kananaskis Lake Upper	Oligotrophic	Prov. Parks	Prov. Parks
Kehiwin Lake	Eutrophic	ALMS	Cyanotoxin Program
Lac La Nonne	Hypereutrophic	ALMS	Cyanotoxin Program
Laurier Lake	Eutrophic	ALMS	Cyanotoxin Program
Long Lake (Near Boyle)	Eutrophic	Prov. Parks	Prov. Parks
Marie Lake	Mesotrophic	ALMS	Cyanotoxin Program
McLeod Lake (East)	Mesotrophic	Prov. Parks	Prov. Parks
Minnie Lake	Mesotrophic	ALMS	Cyanotoxin Program
Miquelon Lake	Mesotrophic	Prov. Parks	Prov. Parks
Moonshine Lake Reservoir	Hypereutrophic	Prov. Parks	Prov. Parks
Moore (Crane) Lake	Oligotrophic	ALMS	Cyanotoxin Program
Nakamun Lake	Hypereutrophic	LTLN	LTLN
Newell Lake Reservoir	Mesotrophic	Prov. Parks	Prov. Parks
Oster Lake	Hypereutrophic	Elk Island Park	Elk Island Park
Pigeon Lake	Eutrophic	Central AB Lakes	Central AB Lakes
Pine Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Reesor Lake Reservoir	Eutrophic	Prov. Parks	Prov. Parks
Sandy Lake	Hypereutrophic	Central AB Lakes	Central AB Lakes
Lac Sante	Mesotrophic	ALMS	Cyanotoxin Program
Saskatoon Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Siler (Stoney) Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Skeleton Lake South Basin	Eutrophic	ALMS	Cyanotoxin Program
Spruce Coulee Reservoir	Oligotrophic	Prov. Parks	Prov. Parks
Steele (Cross) Lake	Eutrophic	Prov. Parks	Prov. Parks
Sturgeon Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Sylvan Lake	Mesotrophic	Central AB Lakes	Central AB Lakes
Twin Valley Reservoir	Eutrophic	Southern L&R	Cyanotoxin Program
Tyrell Lake	Eutrophic	Southern L&R	Cyanotoxin Program
Wapasu Lake	Hypereutrophic	ALMS	Cyanotoxin Program
Winagami Lake	Hypereutrophic	Prov. Parks	Prov. Parks
Wizard Lake	Eutrophic	ALMS	Cyanotoxin Program

Trophic status based on mean summer chlorophyll-a concentration. ALMS = Alberta Lake Management Society's Lakewatch Program; LTLN = Long-term Lake Network Program; Southern L&R = Southern Region Lakes and Reservoirs.

Table A5 List of lakes and reservoirs sampled for anatoxin-a in 2005 including trophic status and parent monitoring program.

Site Name	Trophic Status	Program
Baptiste Lake North Basin	Hypereutrophic	LTLN
Beauvais Lake	Mesotrophic	Prov. Parks
Bluet Lake (Garnier Lake South)	Mesotrophic	ALMS
Crimson Lake	Mesotrophic	Prov. Parks
Dillberry Lake	Mesotrophic	Prov. Parks
Elkwater Lake	Mesotrophic	Prov. Parks
Fishing Lake	Hypereutrophic	ALMS
Fork Lake	Eutrophic	ALMS
Frog Lake	Mesotrophic	ALMS
Garnier Lake North	Mesotrophic	ALMS
Goose Lake	Hypereutrophic	ALMS
Gregoire Lake	Eutrophic	Prov. Parks
Hilda Lake	Mesotrophic	ALMS
Island Lake	Eutrophic	ALMS
Kananaskis Lake Lower	Oligotrophic	Prov. Parks
Kananaskis Lake Upper	Mesotrophic	Prov. Parks
Long Lake	Eutrophic	Prov. Parks
McLeod Lake	Eutrophic	Prov. Parks
Miquelon Lake	Mesotrophic	Prov. Parks
Moonshine Lake Reservoir	Eutrophic	Prov. Parks
Newell Lake Reservoir	Mesotrophic	Prov. Parks
Pine Lake	Eutrophic	ALMS
Reesor Lake Reservoir	Eutrophic	Prov. Parks
Saskatoon Lake	Hypereutrophic	Prov. Parks
Skeleton Lake North Basin	Eutrophic	ALMS
Skeleton Lake South Basin	Eutrophic	ALMS
Spruce Coulee Reservoir	Mesotrophic	Prov. Parks
Steele (Cross) Lake	Hypereutrophic	Prov. Parks
Sturgeon Lake	Hypereutrophic	Prov. Parks
Wabamun Lake East Basin	Eutrophic	LTLN
Wabamun Lake West Basin	Eutrophic	LTLN
Whitefish Lake	Mesotrophic	ALMS
Winagami Lake	Hypereutrophic	Prov. Parks

Trophic status based on mean summer chlorophyll-*a* concentration. ALMS = Alberta Lake Management Society's Lakewatch Program; LTLN = Long-term Lake Network Program.

Appendix III Maximum Microcystin Concentrations in Lakes/Reservoirs

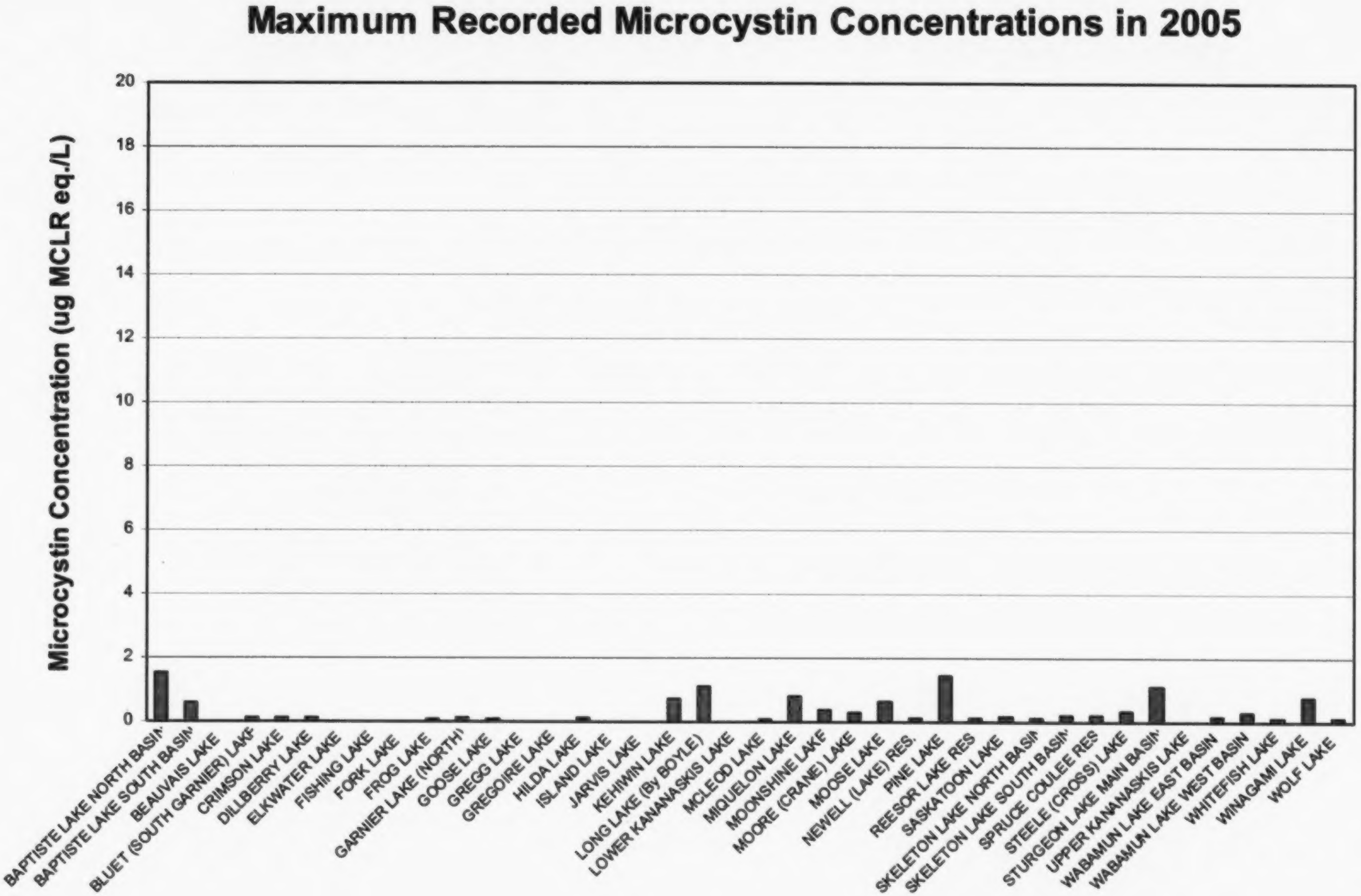

Figure A1 Maximum Microcystin Concentrations in Lakes/Reservoirs Sampled June – September 2005

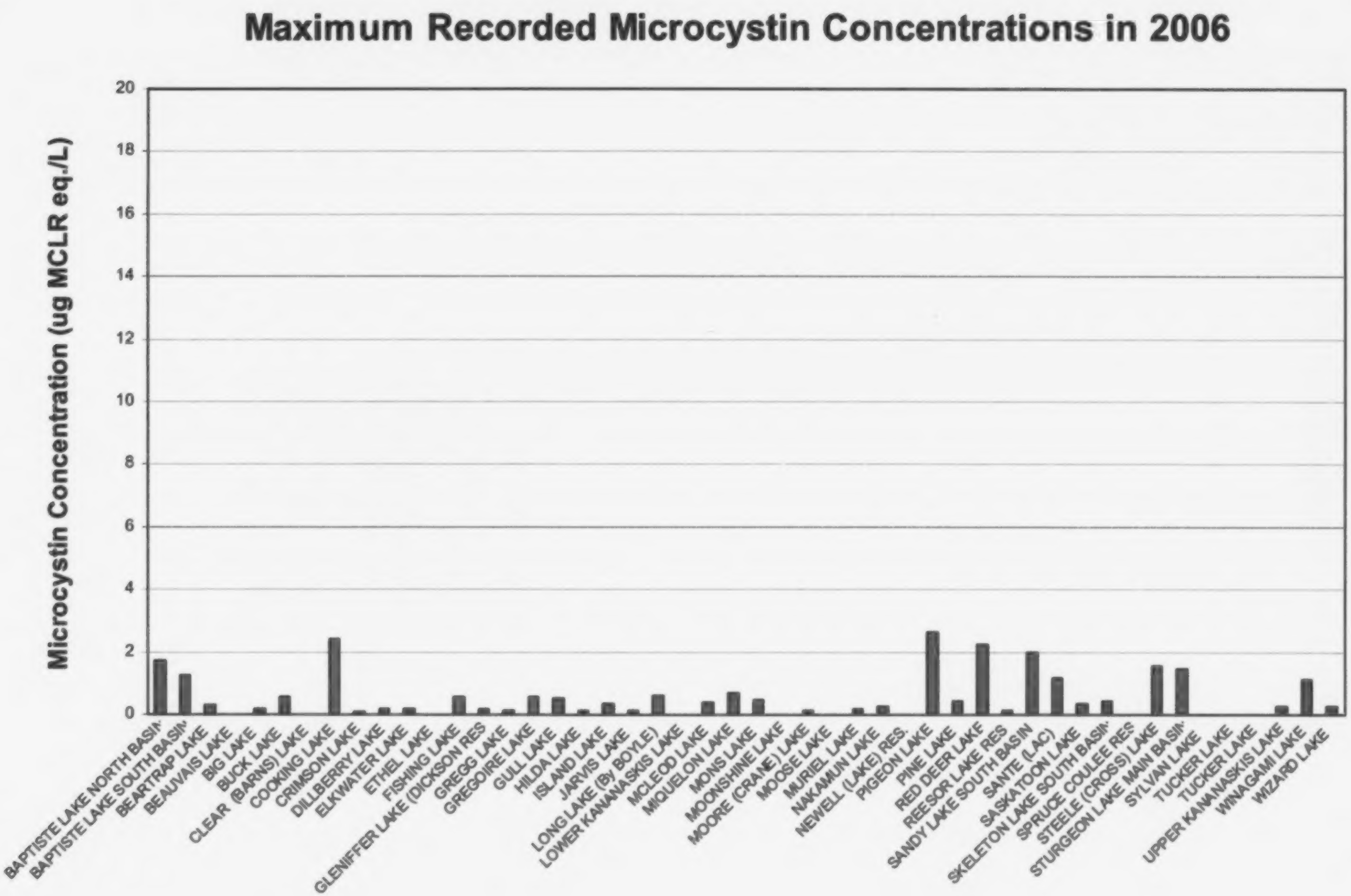

Figure A2 Maximum Microcystin Concentrations in Lakes/Reservoirs Sampled June – September 2006

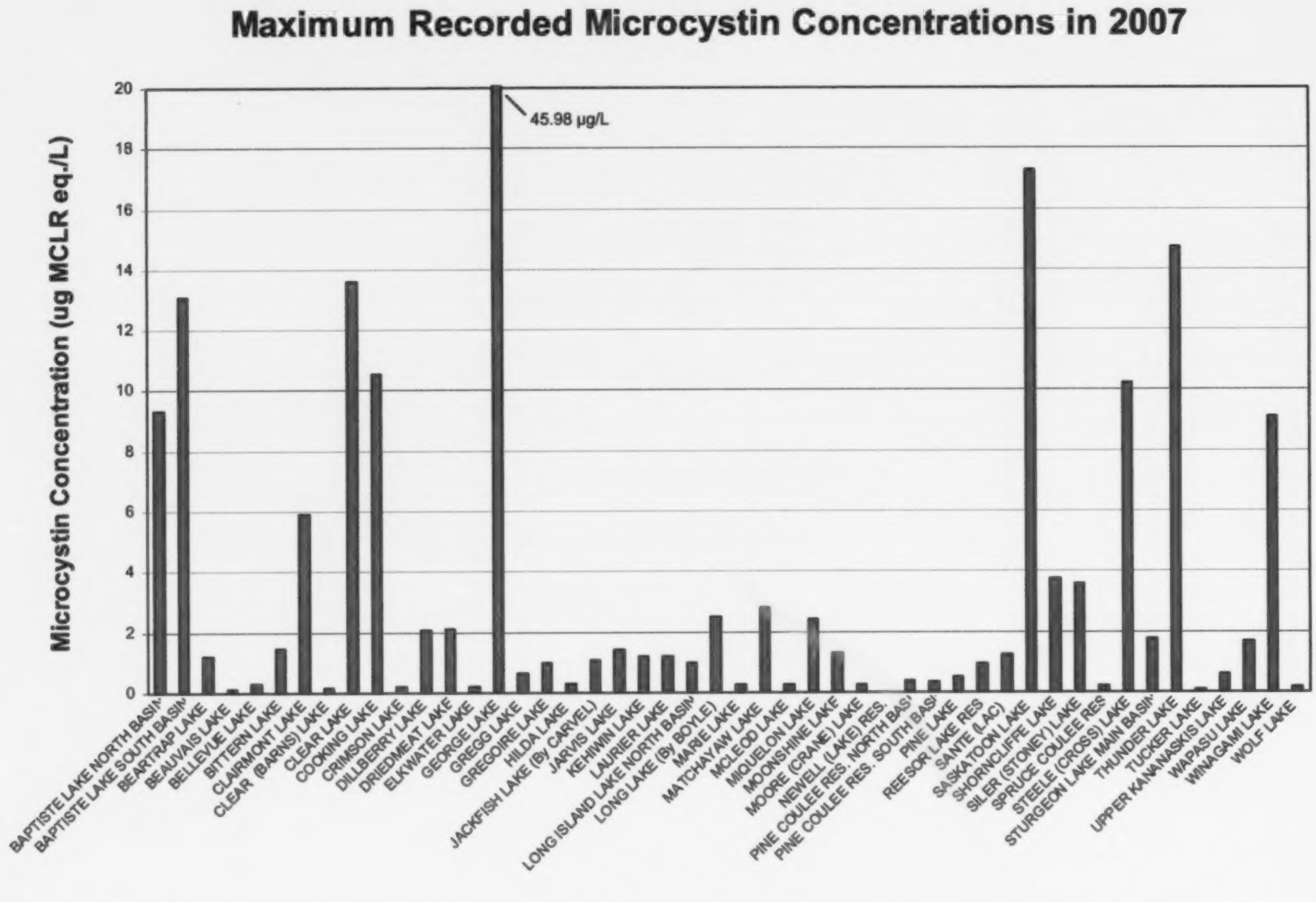

Figure A3 Maximum Microcystin Concentrations in Lakes/Reservoirs Sampled June – September 2007

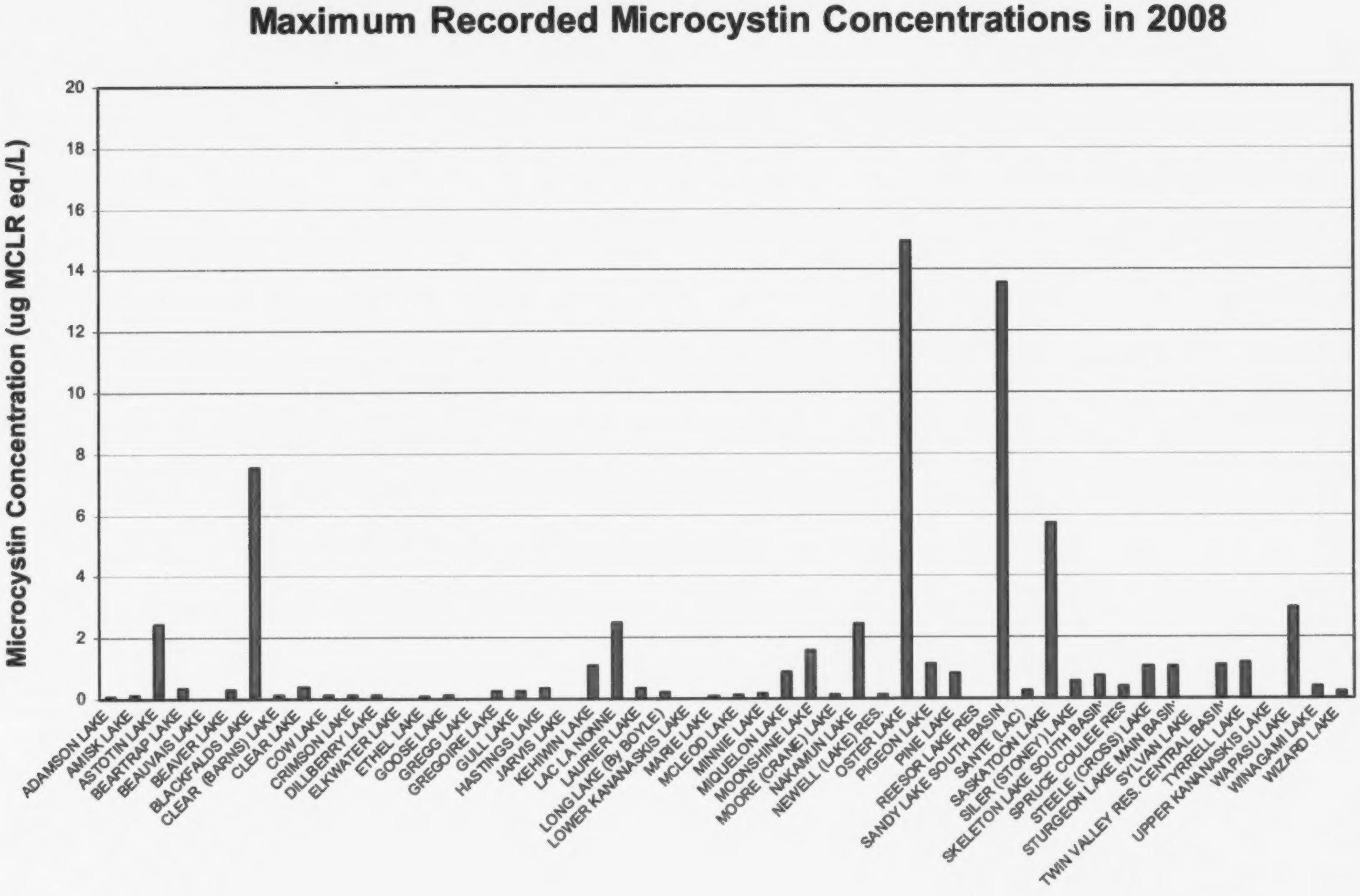

Figure A4 Maximum Microcystin Concentrations in Lakes/Reservoirs Sampled June – September 2008

Appendix IV Quality Assurance/Quality Control

Microcystin Analysis QA/QC

Efforts were made to address aspects of QA/QC pertaining to the analysis of water samples for total microcystin via colorimetric PPI assay. In order to ensure comparability of data over the 4-year period, a standard operating procedure (SOP) developed by AENV was adopted by both laboratories involved in testing. Laboratories were required to use standard measurement units in reporting data. Any changes or modifications of the testing protocol by the laboratories were submitted in advance to the Program Coordinator for review.

As a measure of Laboratory QC, the PPI assay protocol requires that for each sample, 3 subsamples (in 3 microtiter plate test wells) be tested for inhibitory activity to the PP1 enzyme. Values for individual subsamples (wells) are scrutinized and may be disregarded at the discretion of the Laboratory Technician if issues with plating of samples are noted. Sample analysis may be deemed invalid if precision (reproducibility) exceeds %CV >5. These are re-analyzed. The mean of the subsamples is reported as the value for total microcystin.

Lastly, it was desirable to assess whether the inhibitory responses (to the PP1 enzyme) of samples quantified by the PPI assay was due to microcystins. Forty four (44) samples collected during 2007 were submitted to the ACFT Laboratory. Subsamples of each were analyzed by: (1) PPI assay to estimate total microcystin concentration (based on toxicity equivalents to MCLR toxin standards) and (2) LC-MS/MS analysis of MCLR, MCRR, MCYR, MCLW and MCLF concentrations.

Three (7%) samples lacked MCYST as determined by PPI assay (i.e. <0.1 µg MCLReq./L) and LC-MS/MS analysis (Table A6). Microcystin was detected in 41 (93%) of the 44 samples by PPI assay (Table A6). In contrast, only 36 (82%) of the 44 samples analyzed by LC-MS/MS contained detectable concentrations of MCLR (Table A6). Samples analyzed by LC-MS/MS also contained detectable levels of MCRR and MCYR (Table A6), but no MCLW or MCLF (data not shown). Thus in 5 samples, no specific toxin analogues were detected by LC-MS/MS analysis, yet inhibitory responses to PP1 enzyme were noted by PPI assay. Given that more than 70 MCYSTs have been described globally (Zurawell, 2001) - only 5 of which were quantified by LC-MS/MS is this study - it is quite possible the toxic responses (as measured by PPI assay) of these 5 samples may be attributed to unmeasured analogues (see Section 3.2 - Microcystin Analogue Study). However, there remains a remote possibility that a toxic response in the PPI assay may result from the presence of tautomycin - a naturally occurring PP1 inhibitor originating from soil bacteria (*Streptomyces spiroverticillatus*). Although it is highly unlikely that tautomycin occurs in lakes at concentrations required to elicit an inhibitory response (in the PPI assay), this possibility is being addressed.

Table A6 **Results of paired analyses for total MCYST by PPI assay and LC-MS/MS analysis for specific MCYST analogues results for MCLR, MCRR and MCYR.**

Sample	Date	MCYST by PPI (µg MCLReq./L)	MCLR (µg/L)	MCRR (µg/L)	MCYR (µg/L)
Baptiste Lake South	25/06/07	0.36	<0.1	<0.2	<0.1
Baptiste Lake South	18/07/07	0.9	0.6	<0.2	<0.1
Baptiste Lake South	08/08/07	1.56	2.4	0.24	<0.1
Baptiste Lake South	30/08/07	5.2	6.5	<0.2	<0.1
Baptiste Lake South	27/09/07	1.23	1.1	<0.2	<0.1
Baptiste Lake South	29/10/07	<0.07	<0.1	<0.2	<0.1
Baptiste Lake North	18/07/07	1.43	0.8	<0.2	<0.1
Baptiste Lake North	08/08/07	1.42	2.3	<0.2	<0.1
Baptiste Lake North	30/08/07	3.63	4.2	<0.2	<0.1
Baptiste Lake North	27/09/07	2.37	1.9	<0.2	<0.1
Baptiste Lake North	29/10/07	0.19	0.1	<0.2	<0.1
Bittern Lake	11/08/07	0.19	<0.1	<0.2	<0.1
Clairmont Lake	22/10/07	2.45	4.5	<0.2	<0.1
Clairmont Lake	19/09/07	2.45	5.4	<0.2	<0.1
Clear Lake	17/07/07	1.39	1.7	<0.2	<0.1
Clear Lake	29/08/07	11.2	17.2	<0.2	<0.1
Clear Lake	21/09/07	0.89	2.1	<0.2	<0.1
Driedmeat Lake	13/08/07	0.89	2.1	0.89	<0.1
Driedmeat Lake	19/08/07	0.31	0.2	<0.2	<0.1
George Lake	01/08/07	24.33	39.6	<0.2	<0.1
George Lake	23/08/07	6.84	11.6	<0.2	<0.1
Jackfish Lake	15/08/07	0.08	<0.1	<0.2	<0.1
Jackfish Lake	18/08/07	0.36	<0.1	<0.2	<0.1
Miquelon Lake	27/06/07	0.29	<0.1	<0.2	<0.1
Moonshine Lake	31/07/07	0.23	0.6	<0.2	<0.1
Moonshine Lake	23/08/07	0.41	0.5	<0.2	<0.1
Moonshine Lake	23/08/07	0.1	0.2	<0.2	<0.1
Moonshine Lake	18/09/07	<0.07	<0.1	<0.2	<0.1
Moonshine Lake	31/07/07	0.26	0.7	<0.2	<0.1
Saskatoon Lake	02/08/07	3.1	8.9	<0.2	<0.1
Saskatoon Lake	22/08/07	9.8	12.2	<0.2	<0.1
Pine Coulee Res. North	15/08/07	0.31	0.3	<0.2	<0.1
Pine Coulee Res. North	10/07/07	0.08	0.1	<0.2	<0.1
Pine Coulee Res. South	10/07/07	<0.07	<0.1	<0.2	<0.1
Pine Lake	25/07/07	0.26	0.4	<0.2	<0.1
Pine Lake	15/08/07	0.5	0.8	<0.2	<0.1
Shorncliffe Lake	15/08/07	3.68	3.4	<0.2	<0.1
Sturgeon Lake	21/09/07	0.13	0.3	<0.2	<0.1
Thunder Lake	16/07/07	1.09	5.6	1.39	<0.1
Thunder Lake	25/08/07	4.1	13.6	4.89	0.16
Thunder Lake	24/09/07	3.93	9.1	4.12	<0.1
Twin Valley Reservoir	31/07/07	0.51	1.0	<0.2	<0.1
Twin Valley Reservoir	24/09/07	0.16	0.3	<0.2	<0.1
Twin Valley Reservoir	29/08/07	223.5	206.0	19.8	1.1

n.d. = no toxin analogue detected.

Appendix V Raw Data

Table A7 Raw Data 2005

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Baptiste Lake North Basin	19-May-05	<0.1
Baptiste Lake North Basin	16-Jun-05	<0.1
Baptiste Lake North Basin	13-Jul-05	0.25
Baptiste Lake North Basin	16-Aug-05	0.32
Baptiste Lake North Basin	31-Aug-05	0.55
Baptiste Lake North Basin	29-Sep-05	1.52
Baptiste Lake South Basin	19-May-05	<0.1
Baptiste Lake South Basin	16-Jun-05	<0.1
Baptiste Lake South Basin	13-Jul-05	0.17
Baptiste Lake South Basin	16-Aug-05	0.4
Baptiste Lake South Basin	31-Aug-05	0.6
Baptiste Lake South Basin	29-Sep-05	0.24
Beauvais Lake	14-Aug-05	<0.1
Bluet Lake (Garnier Lake South)	16-Jun-05	0.11
Bluet Lake (Garnier Lake South)	7-Jul-05	<0.1
Bluet Lake (Garnier Lake South)	28-Jul-05	0.11
Bluet Lake (Garnier Lake South)	18-Aug-05	0.1
Bluet Lake (Garnier Lake South)	8-Sep-05	0.1
Crimson Lake	3-Jul-05	<0.1
Crimson Lake	19-Jul-05	0.13
Crimson Lake	1-Aug-05	<0.1
Crimson Lake	29-Aug-05	<0.1
Crimson Lake	5-Sep-05	<0.1
Dillberry Lake	28-Jun-05	<0.1
Dillberry Lake	26-Jul-05	0.12
Dillberry Lake	9-Aug-05	<0.1
Dillberry Lake	29-Aug-05	<0.1
Dillberry Lake	14-Sep-05	<0.1
Elkwater Lake	14-Jul-05	<0.1
Elkwater Lake	23-Jul-05	<0.1
Elkwater Lake	7-Aug-05	<0.1
Elkwater Lake	5-Sep-05	<0.1
Fishing Lake	30-Jul-05	<0.1

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Fishing Lake	29-Aug-05	<0.1
Fork Lake	8-Jun-05	<0.1
Fork Lake	27-Jun-05	<0.1
Fork Lake	19-Jul-05	<0.1
Fork Lake	9-Aug-05	<0.1
Fork Lake	1-Sep-05	<0.1
Fork Lake	20-Sep-05	<0.1
Frog Lake	30-Jul-05	0.1
Frog Lake	29-Aug-05	<0.1
Garnier Lake North	16-Jun-05	0.1
Garnier Lake North	7-Jul-05	0.13
Garnier Lake North	28-Jul-05	0.12
Garnier Lake North	18-Aug-05	0.14
Garnier Lake North	8-Sep-05	<0.1
Goose Lake	3-Jul-05	0.1
Goose Lake	6-Aug-05	<0.1
Goose Lake	27-Aug-05	<0.1
Goose Lake	25-Sep-05	<0.1
Gregg Lake	12-Aug-05	<0.1
Gregoire Lake	28-Jun-05	<0.1
Gregoire Lake	12-Jul-05	<0.1
Gregoire Lake	29-Jul-05	<0.1
Gregoire Lake	8-Aug-05	<0.1
Gregoire Lake	21-Aug-05	<0.1
Gregoire Lake	5-Sep-05	<0.1
Hilda Lake	27-Jun-05	0.12
Hilda Lake	28-Jul-05	0.12
Hilda Lake	28-Aug-05	<0.1
Hilda Lake	11-Sep-05	0.13
Island Lake	9-Jun-05	<0.1
Island Lake	30-Jun-05	<0.1
Island Lake	21-Jul-05	<0.1
Island Lake	13-Aug-05	<0.1

Table A7 Raw Data 2005

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Island Lake	30-Aug-05	<0.1
Island Lake	22-Sep-05	<0.1
Jarvis Lake	12-Aug-05	<0.1
Kananaskis Lake Lower	29-Jun-05	<0.1
Kananaskis Lake Lower	25-Jul-05	<0.1
Kananaskis Lake Lower	21-Sep-05	<0.1
Kananaskis Lake Upper	29-Jun-05	<0.1
Kananaskis Lake Upper	25-Jul-05	<0.1
Kananaskis Lake Upper	21-Sep-05	<0.1
Kehiwin Lake	25-Jun-05	<0.1
Kehiwin Lake	17-Jul-05	0.73
Kehiwin Lake	21-Aug-05	<0.1
Long Lake (by Boyle)	7-Jun-05	<0.1
Long Lake (by Boyle)	28-Jun-05	<0.1
Long Lake (by Boyle)	19-Jul-05	<0.1
Long Lake (by Boyle)	26-Jul-05	<0.1
Long Lake (by Boyle)	9-Aug-05	0.46
Long Lake (by Boyle)	28-Aug-05	0.42
Long Lake (by Boyle)	30-Aug-05	1.06
Long Lake (by Boyle)	20-Sep-05	1.12
McLeod Lake (East)	26-Jun-05	<0.1
McLeod Lake (East)	17-Jul-05	<0.1
McLeod Lake (East)	21-Aug-05	0.1
McLeod Lake (East)	30-Aug-05	<0.1
Miquelon Lake	3-Aug-05	<0.1
Miquelon Lake	19-Aug-05	<0.1
Miquelon Lake	15-Sep-05	0.82
Moonshine Lake Reservoir	27-Jun-05	<0.1
Moonshine Lake Reservoir	27-Jul-05	0.39
Moonshine Lake Reservoir	9-Aug-05	0.34
Moonshine Lake Reservoir	23-Aug-05	0.12
Moonshine Lake Reservoir	26-Sep-05	<0.1
Moore (Crane) Lake	11-Jun-05	0.19

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Moore (Crane) Lake	6-Jul-05	0.29
Moore (Crane) Lake	29-Jul-05	0.1
Moore (Crane) Lake	21-Aug-05	<0.1
Moore (Crane) Lake	18-Sep-05	0.14
Moose Lake	20-Jun-05	0.29
Moose Lake	9-Jul-05	0.65
Moose Lake	4-Aug-05	0.5
Moose Lake	4-Sep-05	0.23
Newell Lake Reservoir	6-Jul-05	0.13
Newell Lake Reservoir	27-Jul-05	<0.1
Newell Lake Reservoir	15-Aug-05	<0.1
Newell Lake Reservoir	31-Aug-05	<0.1
Pine Lake	24-Jun-05	0.56
Pine Lake	15-Jul-05	0.62
Pine Lake	5-Aug-05	1.2
Pine Lake	26-Aug-05	0.56
Pine Lake	16-Sep-05	1.44
Reesor Lake Reservoir	19-Jun-05	<0.1
Reesor Lake Reservoir	15-Jul-05	<0.1
Reesor Lake Reservoir	5-Aug-05	0.1
Reesor Lake Reservoir	7-Sep-05	<0.1
Reesor Lake Reservoir	28-Sep-05	0.13
Saskatoon Lake	25-Jul-05	0.13
Saskatoon Lake	8-Aug-05	0.17
Saskatoon Lake	25-Aug-05	0.15
Saskatoon Lake	26-Sep-05	<0.1
Skeleton Lake North Basin	29-Jun-05	<0.1
Skeleton Lake North Basin	29-Jun-05	<0.1
Skeleton Lake North Basin	23-Jul-05	<0.1
Skeleton Lake North Basin	16-Aug-05	0.13
Skeleton Lake South Basin	10-Jun-05	0.1
Skeleton Lake South Basin	29-Jun-05	0.11
Skeleton Lake South Basin	31-Jul-05	0.15

Table A7 Raw Data 2005

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Skeleton Lake South Basin	16-Aug-05	0.18
Skeleton Lake South Basin	2-Sep-05	0.2
Spruce Coulee Reservoir	19-Jun-05	<0.1
Spruce Coulee Reservoir	5-Aug-05	0.21
Spruce Coulee Reservoir	7-Sep-05	0.19
Spruce Coulee Reservoir	28-Sep-05	<0.1
Steele (Cross) Lake	28-Jun-05	<0.1
Steele (Cross) Lake	27-Jul-05	<0.1
Steele (Cross) Lake	16-Aug-05	0.35
Steele (Cross) Lake	31-Aug-05	<0.1
Steele (Cross) Lake	21-Sep-05	<0.1
Sturgeon Lake	28-Jul-05	0.34
Sturgeon Lake	25-Aug-05	1.12
Wabamun Lake East Basin	21-Jul-05	0.11
Wabamun Lake East Basin	16-Aug-05	0.13
Wabamun Lake East Basin	23-Aug-05	0.16
Wabamun Lake East Basin	15-Sep-05	0.11
Wabamun Lake West Basin	21-Jul-05	<0.1
Wabamun Lake West Basin	16-Aug-05	0.3
Wabamun Lake West Basin	23-Aug-05	0.16
Wabamun Lake West Basin	15-Sep-05	0.12
Whitefish Lake	5-Jun-05	0.14
Whitefish Lake	10-Jul-05	<0.1
Whitefish Lake	31-Jul-05	<0.1
Whitefish Lake	20-Aug-05	0.11
Winagami Lake	23-Jun-05	0.21
Winagami Lake	21-Jul-05	0.5
Winagami Lake	4-Aug-05	0.76
Winagami Lake	24-Aug-05	0.56
Winagami Lake	1-Sep-05	0.29
Wolf Lake	15-Jun-05	<0.1
Wolf Lake	4-Aug-05	0.11
Wolf Lake	20-Aug-05	0.13

Table A8 Raw Data 2006

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Baptiste Lake North Basin	25-Jul-06	0.67
Baptiste Lake North Basin	9-Aug-06	1.72
Baptiste Lake North Basin	10-Sep-06	1.36
Baptiste Lake North Basin	26-Sep-06	1.54
Baptiste Lake South Basin	25-Jul-06	0.69
Baptiste Lake South Basin	9-Aug-06	1.23
Baptiste Lake South Basin	10-Sep-06	1.15
Baptiste Lake South Basin	26-Sep-06	0.90
Bear Trap Lake	24-Jul-06	0.16
Bear Trap Lake	18-Aug-06	<0.1
Bear Trap Lake	5-Sep-06	0.30
Beauvais Lake	13-Aug-06	<0.1
Beauvais Lake	27-Aug-06	<0.1
Big Lake	21-Jul-06	<0.1
Big Lake	11-Aug-06	0.1
Big Lake	1-Sep-06	0.12
Big Lake	22-Sep-06	0.17
Buck Lake	24-Jul-06	0.16
Buck Lake	21-Aug-06	0.44
Buck Lake	28-Sep-06	0.58
Clear (Barnes) Lake	14-Jul-06	<0.1
Clear (Barnes) Lake	13-Aug-06	<0.1
Clear (Barnes) Lake	8-Sep-06	<0.1
Clear (Barnes) Lake	29-Sep-06	<0.1
Cooking Lake	26-Jul-06	2.23
Cooking Lake	9-Aug-06	1.94
Cooking Lake	23-Aug-06	2.41
Cooking Lake	11-Sep-06	1.38
Crimson Lake	11-Jul-06	0.10
Dillberry Lake	14-Jul-06	<0.1
Dillberry Lake	16-Aug-06	<0.1
Dillberry Lake	6-Sep-06	0.11
Dillberry Lake	29-Sep-06	<0.1

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Elkwater Lake	11-Jul-06	<0.1
Elkwater Lake	1-Aug-06	0.19
Elkwater Lake	22-Aug-06	0.17
Elkwater Lake	29-Sep-06	<0.1
Ethel Lake	16-May-06	<0.1
Ethel Lake	13-Jun-06	<0.1
Ethel Lake	12-Jul-06	<0.1
Ethel Lake	1-Aug-06	<0.1
Ethel Lake	16-Aug-06	<0.1
Ethel Lake	22-Sep-06	<0.1
Ethel Lake	12-Oct-06	<0.1
Fishing Lake	6-Jul-06	0.55
Fishing Lake	27-Jul-06	<0.1
Fishing Lake	7-Sep-06	0.13
Frog Lake	6-Jul-06	<0.1
Frog Lake	7-Sep-06	<0.1
Gleniffer Lake (Dickson Dam Res.)	27-Jul-06	<0.1
Gleniffer Lake (Dickson Dam Res.)	22-Aug-06	0.16
Gleniffer Lake (Dickson Dam Res.)	19-Sep-06	0.13
Gregg Lake	24-Jul-06	0.12
Gregg Lake	14-Aug-06	<0.1
Gregg Lake	4-Sep-06	0.13
Gregg Lake	28-Sep-06	<0.1
Gregoire Lake	25-Jul-06	0.56
Gregoire Lake	11-Aug-06	<0.1
Gregoire Lake	29-Aug-06	0.14
Gregoire Lake	20-Sep-06	<0.1
Gull Lake	18-Jul-06	<0.1
Gull Lake	26-Jul-06	0.26
Gull Lake	19-Aug-06	0.13
Gull Lake	2-Sep-06	<0.1
Gull Lake	6-Sep-06	0.27
Gull Lake	27-Sep-06	0.52

Table A8 Raw Data 2006

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Hilda Lake	15-Jul-06	<0.1
Hilda Lake	1-Aug-06	0.11
Hilda Lake	21-Aug-06	<0.1
Hilda Lake	11-Sep-06	<0.1
Island Lake	14-Aug-06	0.33
Jarvis Lake	24-Jul-06	<0.1
Jarvis Lake	14-Aug-06	<0.1
Jarvis Lake	4-Sep-06	<0.1
Jarvis Lake	28-Sep-06	0.12
Kananaskis Lower	22-Jul-06	<0.1
Kananaskis Lower	18-Aug-06	<0.1
Kananaskis Lower	7-Sep-06	<0.1
Kananaskis Lower	26-Sep-06	<0.1
Kananaskis Upper	22-Jul-06	0.27
Kananaskis Upper	18-Aug-06	<0.1
Kananaskis Upper	7-Sep-06	<0.1
Kananaskis Upper	26-Sep-06	<0.1
Long Lake (by Boyle)	21-Jul-06	0.22
Long Lake (by Boyle)	8-Aug-06	0.61
Long Lake (by Boyle)	25-Aug-06	0.41
Long Lake (by Boyle)	27-Sep-06	0.44
McLeod Lake (East)	23-Jun-06	<0.1
McLeod Lake (East)	24-Jul-06	<0.1
McLeod Lake (East)	13-Aug-06	<0.1
McLeod Lake (East)	28-Aug-06	<0.1
McLeod Lake (East)	5-Sep-06	0.38
Miquelon Lake	19-Jul-06	0.70
Miquelon Lake	17-Aug-06	0.42
Miquelon Lake	30-Aug-06	0.28
Miquelon Lake	21-Sep-06	0.45
Mons Lake	19-Jul-06	0.11
Mons Lake	5-Aug-06	0.10
Mons Lake	26-Aug-06	0.40

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Mons Lake	23-Sep-06	0.48
Moonshine Lake Reservoir	25-Jun-06	<0.1
Moonshine Lake Reservoir	11-Jul-06	<0.1
Moonshine Lake Reservoir	8-Aug-06	<0.1
Moonshine Lake Reservoir	28-Aug-06	<0.1
Moore (Crane) Lake	15-Jul-06	0.14
Moore (Crane) Lake	31-Jul-06	0.13
Moore (Crane) Lake	21-Aug-06	<0.1
Moose Lake	10-Jul-06	<0.1
Moose Lake	3-Aug-06	<0.1
Moose Lake	18-Aug-06	<0.1
Moose Lake	5-Sep-06	<0.1
Muriel Lake	14-Aug-06	0.18
Nakamun Lake	10-May-06	<0.1
Nakamun Lake	7-Jun-06	<0.1
Nakamun Lake	5-Jul-06	<0.1
Nakamun Lake	25-Jul-06	0.14
Nakamun Lake	17-Aug-06	0.23
Nakamun Lake	20-Sep-06	0.25
Nakamun Lake	18-Oct-06	<0.1
Newell Lake Reservoir	19-Jul-06	<0.1
Pigeon Lake	20-Jul-06	0.63
Pigeon Lake	17-Aug-06	0.28
Pigeon Lake	12-Sep-06	2.62
Pine Lake	12-Jul-06	0.14
Pine Lake	12-Aug-06	0.37
Pine Lake	23-Aug-06	0.44
Pine Lake	14-Sep-06	0.22
Red Deer Lake	27-Jul-06	0.97
Red Deer Lake	16-Aug-06	1.50
Red Deer Lake	21-Sep-06	2.24
Reesor Lake Reservoir	11-Jun-06	<0.1
Reesor Lake Reservoir	11-Jul-06	<0.1

Table A8 Raw Data 2006

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Reesor Lake Reservoir	1-Aug-06	0.11
Reesor Lake Reservoir	22-Aug-06	<0.1
Reesor Lake Reservoir	29-Sep-06	0.11
Sandy Lake	20-Jul-06	0.64
Sandy Lake	10-Aug-06	1.89
Sandy Lake	31-Aug-06	1.98
Sandy Lake	20-Sep-06	1.70
Lac Sante	7-Jul-06	0.42
Lac Sante	5-Aug-06	0.31
Lac Sante	27-Aug-06	0.74
Lac Sante	23-Sep-06	1.18
Saskatoon Lake	26-Jun-06	<0.1
Saskatoon Lake	10-Jul-06	0.34
Saskatoon Lake	13-Aug-06	0.14
Saskatoon Lake	28-Aug-06	0.20
Saskatoon Lake	24-Sep-06	0.15
Skeleton Lake South Basin	21-Jul-06	<0.1
Skeleton Lake South Basin	8-Aug-06	<0.1
Skeleton Lake South Basin	28-Aug-06	0.20
Skeleton Lake South Basin	19-Sep-06	0.44
Spruce Coulee Reservoir	12-Jun-06	<0.1
Spruce Coulee Reservoir	11-Jul-06	<0.1
Spruce Coulee Reservoir	1-Aug-06	<0.1
Spruce Coulee Reservoir	22-Aug-06	<0.1
Spruce Coulee Reservoir	29-Sep-06	<0.1
Steele (Cross) Lake	13-Jul-06	0.34
Steele (Cross) Lake	2-Aug-06	0.17
Steele (Cross) Lake	28-Aug-06	1.55
Steele (Cross) Lake	18-Sep-06	1.00
Sturgeon Lake	24-Jul-06	0.22
Sturgeon Lake	13-Aug-06	0.17
Sturgeon Lake	27-Aug-06	1.45
Sylvan Lake	2-Aug-06	<0.1
Sylvan Lake	29-Aug-06	<0.1
Sylvan Lake	19-Sep-06	<0.1
Tucker Lake	15-Jul-06	<0.1
Tucker Lake	1-Aug-06	<0.1
Tucker Lake	22-Aug-06	<0.1
Tucker Lake	6-Sep-06	<0.1
Winagami Lake	19-Jun-06	<0.1
Winagami Lake	1-Aug-06	0.59
Winagami Lake	22-Aug-06	1.12
Winagami Lake	27-Sep-06	0.25
Wizard Lake	12-Jul-06	<0.1
Wizard Lake	31-Jul-06	<0.1
Wizard Lake	21-Aug-06	0.25
Wizard Lake	13-Sep-06	0.16
Wolf Lake	17-Jul-06	<0.1
Wolf Lake	10-Aug-06	<0.1
Wolf Lake	11-Sep-06	<0.1

Table A9 Raw Data 2007

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Baptiste Lake South Basin	15-May-07	<0.1
Baptiste Lake South Basin	25-Jun-07	0.1
Baptiste Lake South Basin	18-Jul-07	1.13
Baptiste Lake South Basin	8-Aug-07	4.94
Baptiste Lake South Basin	30-Aug-07	13.08
Baptiste Lake South Basin	27-Sep-07	3.75
Baptiste Lake South Basin	29-Oct-07	0.12
Baptiste Lake North Basin	15-May-07	<0.1
Baptiste Lake North Basin	25-Jun-07	1.7
Baptiste Lake North Basin	18-Jul-07	0.87
Baptiste Lake North Basin	8-Aug-07	4.1
Baptiste Lake North Basin	30-Aug-07	9.35
Baptiste Lake North Basin	27-Sep-07	5.44
Baptiste Lake North Basin	29-Oct-07	0.41
Bear Trap Lake	27-Jun-07	0.16
Bear Trap Lake	17-Jul-07	0.12
Bear Trap Lake	8-Aug-07	1.23
Bear Trap Lake	6-Sep-07	0.24
Beauvais Lake	29-Jul-07	0.15
Beauvais Lake	12-Aug-07	0.1
Beauvais Lake	3-Sep-07	<0.1
Beauvais Lake	30-Sep-07	<0.1
Lac Bellevue	1-Jul-07	0.19
Lac Bellevue	17-Jul-07	0.22
Lac Bellevue	18-Aug-07	0.32
Bittern Lake	10-Jul-07	0.32
Bittern Lake	14-Aug-07	1.47
Clairmont Lake	22-Aug-07	5.91
Clairmont Lake	19-Sep-07	0.27
Clear Lake	12-Jul-07	0.76
Clear Lake	29-Aug-07	13.6
Clear Lake	21-Sep-07	0.83
Clear (Barnes) Lake	4-Jul-07	<0.1

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Clear (Barnes) Lake	24-Jul-07	0.19
Clear (Barnes) Lake	16-Sep-07	0.12
Cooking Lake	27-Jul-07	10.44
Cooking Lake	9-Aug-07	10.54
Cooking Lake	26-Aug-07	6.17
Cooking Lake	3-Oct-07	4.25
Crimson Lake	6-Jul-07	<0.1
Crimson Lake	26-Jul-07	0.16
Crimson Lake	21-Aug-07	0.23
Crimson Lake	9-Sep-07	<0.1
Dillberry Lake	27-Jul-07	0.26
Dillberry Lake	24-Aug-07	2.09
Dillberry Lake	12-Sep-07	0.48
Driedmeat Lake	11-Jul-07	<0.1
Driedmeat Lake	13-Aug-07	2.11
Driedmeat Lake	19-Sep-07	0.5
Eagle Lake	2-Aug-07	1.89
Elkwater Lake	12-Aug-07	0.21
Elkwater Lake	13-Sep-07	<0.1
George Lake	12-Jul-07	1.09
George Lake	1-Aug-07	45.98
George Lake	23-Aug-07	21.82
George Lake	18-Sep-07	19.31
Gregg Lake	18-Jul-07	0.18
Gregg Lake	9-Aug-07	0.63
Gregg Lake	30-Aug-07	<0.1
Gregoire Lake	27-Jun-07	<0.1
Gregoire Lake	7-Aug-07	1.01
Gregoire Lake	27-Aug-07	<0.1
Gregoire Lake	19-Sep-07	<0.1
Hilda Lake	10-Jul-07	<0.1
Hilda Lake	15-Aug-07	0.3
Hilda Lake	29-Aug-07	<0.1

Table A9 Raw Data 2007

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Hilda Lake	20-Sep-07	0.17
Jackfish Lake (by Carvel)	16-Jul-07	<0.1
Jackfish Lake (by Carvel)	15-Aug-07	0.44
Jackfish Lake (by Carvel)	18-Sep-07	1.1
Jarvis Lake	17-Jul-07	<0.1
Jarvis Lake	9-Aug-07	1.41
Jarvis Lake	30-Aug-07	<0.1
Kananaskis Lake Upper	24-Jul-07	<0.1
Kananaskis Lake Upper	7-Aug-07	0.62
Kananaskis Lake Upper	21-Aug-07	0.13
Kananaskis Lake Upper	18-Sep-07	<0.1
Kehiwin Lake	30-Jun-07	0.16
Kehiwin Lake	18-Jul-07	<0.1
Kehiwin Lake	31-Aug-07	0.74
Kehiwin Lake	29-Sep-07	1.21
Laurier Lake	29-Jun-07	0.23
Laurier Lake	18-Jul-07	<0.1
Laurier Lake	8-Aug-07	1.21
Laurier Lake	6-Sep-07	0.16
Long Island Lake North Basin	25-Jul-07	1.01
Long Island Lake North Basin	8-Aug-07	<0.1
Long Island Lake North Basin	5-Sep-07	0.74
Long Island Lake South Basin	25-Jul-07	0.34
Long Island Lake South Basin	8-Aug-07	<0.1
Long Island Lake South Basin	5-Sep-07	0.1
Long Lake (by Boyle)	25-Jun-07	0.31
Long Lake (by Boyle)	23-Jul-07	1.09
Long Lake (by Boyle)	12-Aug-07	1.33
Long Lake (by Boyle)	26-Aug-07	2.52
Long Lake (by Boyle)	26-Sep-07	1.39
Marie Lake	12-Jul-07	<0.1
Marie Lake	14-Aug-07	0.24
Marie Lake	19-Sep-07	0.1

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Matchayaw Lake	16-Jul-07	0.25
Matchayaw Lake	7-Aug-07	<0.1
Matchayaw Lake	27-Aug-07	2.8
Matchayaw Lake	4-Oct-07	0.14
McLeod Lake (East)	26-Jul-07	0.25
McLeod Lake (East)	16-Aug-07	0.21
Miquelon Lake	27-Jun-07	0.33
Miquelon Lake	25-Jul-07	<0.1
Miquelon Lake	9-Aug-07	2.27
Miquelon Lake	30-Aug-07	2.4
Moonshine Lake Reservoir	12-Jul-07	<0.1
Moonshine Lake Reservoir	31-Jul-07	1.28
Moonshine Lake Reservoir	23-Aug-07	0.33
Moonshine Lake Reservoir	18-Sep-07	<0.1
Moore (Crane) Lake	10-Jul-07	0.28
Moore (Crane) Lake	3-Aug-07	<0.1
Moore (Crane) Lake	29-Aug-07	0.1
Moore (Crane) Lake	20-Sep-07	0.13
Newell Lake Reservoir	18-Jul-07	<0.1
Pine Coulee Res South Basin	16-Jul-07	0.35
Pine Coulee Res North Basin	16-Jul-07	0.41
Pine Lake	25-Jul-07	0.4
Pine Lake	15-Aug-07	0.51
Reesor Lake Reservoir	5-Jul-07	0.64
Reesor Lake Reservoir	12-Aug-07	0.97
Reesor Lake Reservoir	13-Sep-07	<0.1
Lac Sante	1-Jul-07	0.1
Lac Sante	18-Aug-07	1.26
Saskatoon Lake	13-Jul-07	2.25
Saskatoon Lake	2-Aug-07	17.29
Saskatoon Lake	22-Aug-07	13.6
Saskatoon Lake	20-Sep-07	9.53
Shorncliffe Lake	12-Jul-07	<0.1

Table A9 Raw Data 2007

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Shorncliffe Lake	15-Aug-07	3.77
Shorncliffe Lake	20-Sep-07	2.14
Siler (Stoney) Lake	28-Jun-07	0.11
Siler (Stoney) Lake	16-Jul-07	3.57
Siler (Stoney) Lake	9-Aug-07	2.16
Siler (Stoney) Lake	13-Sep-07	1.92
Spruce Coulee Reservoir	5-Jul-07	0.16
Spruce Coulee Reservoir	12-Aug-07	0.21
Spruce Coulee Reservoir	13-Sep-07	0.15
Steele (Cross) Lake	24-Jul-07	0.6
Steele (Cross) Lake	14-Aug-07	10.25
Steele (Cross) Lake	29-Aug-07	5.21
Sturgeon Lake	11-Jul-07	0.85
Sturgeon Lake	24-Aug-07	1.79
Sturgeon Lake	21-Sep-07	0.22
Thunder Lake	16-Jul-07	<0.1
Thunder Lake	28-Aug-07	14.74
Thunder Lake	24-Sep-07	8.81
Tucker Lake	11-Jul-07	<0.1
Tucker Lake	31-Aug-07	<0.1
Tucker Lake	19-Sep-07	0.1
Twin Valley Reservoir	24-Sep-07	<0.1
Wapasu Lake	4-Jul-07	0.4
Wapasu Lake	28-Aug-07	1.7
Wapasu Lake	27-Sep-07	0.68
Winagami Lake	25-Aug-07	9.1
Winagami Lake	21-Sep-07	0.17
Wolf Lake	2-Aug-07	0.17
Wolf Lake	30-Aug-07	<0.1
Wolf Lake	18-Sep-07	<0.1

Table A10 Raw Data 2008

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Adamson Lake	25-Jun-08	<0.1
Adamson Lake	24-Jul-08	<0.1
Adamson Lake	13-Aug-08	<0.1
Adamson Lake	9-Sep-08	0.1
Adamson Lake	7-Oct-08	<0.1
Amisk Lake	4-Jun-08	0.15
Amisk Lake	30-Jun-08	0.14
Amisk Lake	23-Jul-08	<0.1
Amisk Lake	12-Aug-08	0.13
Amisk Lake	14-Sep-08	<0.1
Astotin Lake	26-Jun-08	1.04
Astotin Lake	22-Jul-08	1.21
Astotin Lake	14-Aug-08	1.98
Astotin Lake	8-Sep-08	2.45
Astotin Lake	2-Oct-08	<0.1
Bear Trap Lake	11-Jun-08	0.37
Bear Trap Lake	17-Jul-08	0.24
Bear Trap Lake	5-Aug-08	0.17
Bear Trap Lake	25-Aug-08	0.14
Bear Trap Lake	15-Sep-08	0.13
Beauvais Lake	12-Jun-08	<0.1
Beauvais Lake	10-Jul-08	<0.1
Beauvais Lake	31-Jul-08	<0.1
Beauvais Lake	18-Aug-08	<0.1
Beauvais Lake	18-Sep-08	<0.1
Beaver Lake	4-Jun-08	0.14
Beaver Lake	27-Jun-08	0.15
Beaver Lake	23-Jul-08	<0.1
Beaver Lake	19-Aug-08	0.29
Beaver Lake	14-Sep-08	<0.1
Blackfalds Lake	28-Jun-08	0.76
Blackfalds Lake	19-Jul-08	7.55
Blackfalds Lake	23-Sep-08	<0.1

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Clear Lake	29-Jul-08	<0.1
Clear Lake	28-Aug-08	0.38
Clear Lake	1-Oct-08	<0.1
Clear (Barnes) Lake	26-Jun-08	0.11
Clear (Barnes) Lake	29-Jul-08	<0.1
Clear (Barnes) Lake	19-Aug-08	0.12
Clear (Barnes) Lake	8-Sep-08	<0.1
Cow Lake	23-Jul-08	<0.1
Cow Lake	25-Aug-08	<0.1
Cow Lake	18-Sep-08	0.15
Crimson Lake	21-Jul-08	<0.1
Crimson Lake	14-Aug-08	<0.1
Crimson Lake	28-Aug-08	0.15
Crimson Lake	8-Sep-08	<0.1
Dillberry Lake	13-Jun-08	<0.1
Dillberry Lake	13-Jul-08	0.12
Dillberry Lake	8-Aug-08	<0.1
Dillberry Lake	2-Sep-08	<0.1
Dillberry Lake	1-Oct-08	<0.1
Elkwater Lake	23-Jun-08	<0.1
Elkwater Lake	14-Jul-08	<0.1
Elkwater Lake	29-Aug-08	<0.1
Elkwater Lake	20-Sep-08	<0.1
Ethel Lake	27-May-08	<0.1
Ethel Lake	24-Jun-08	<0.1
Ethel Lake	15-Jul-08	<0.1
Ethel Lake	6-Aug-08	<0.1
Ethel Lake	25-Aug-08	<0.1
Ethel Lake	18-Sep-08	0.1
Ethel Lake	15-Oct-08	<0.1
Goose Lake	24-Jun-08	0.13
Goose Lake	14-Jul-08	<0.1
Goose Lake	11-Aug-08	<0.1

Table A10 Raw Data 2008

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Goose Lake	9-Sep-08	0.14
Goose Lake	28-Sep-08	<0.1
Gregg Lake	9-Jun-08	<0.1
Gregg Lake	7-Jul-08	<0.1
Gregg Lake	5-Aug-08	<0.1
Gregg Lake	25-Aug-08	<0.1
Gregg Lake	16-Sep-08	<0.1
Gregoire Lake	18-Jun-08	0.10
Gregoire Lake	14-Jul-08	0.27
Gregoire Lake	6-Aug-08	<0.1
Gregoire Lake	4-Sep-08	0.1
Gregoire Lake	29-Sep-08	0.14
Gull Lake	23-Jul-08	0.28
Gull Lake	20-Aug-08	0.27
Gull Lake	17-Sep-08	0.16
Hastings Lake	21-Jun-08	0.36
Hastings Lake	16-Jul-08	0.27
Hastings Lake	1-Sep-08	0.13
Hastings Lake	27-Sep-08	0.1
Jarvis Lake	9-Jun-08	<0.1
Jarvis Lake	7-Jul-08	<0.1
Jarvis Lake	5-Aug-08	<0.1
Jarvis Lake	25-Aug-08	<0.1
Jarvis Lake	16-Sep-08	<0.1
Kananaskis Lake Lower	8-Jul-08	<0.1
Kananaskis Lake Lower	29-Jul-08	<0.1
Kananaskis Lake Lower	21-Aug-08	<0.1
Kananaskis Lake Lower	16-Sep-08	<0.1
Kananaskis Lake Upper	8-Jul-08	<0.1
Kananaskis Lake Upper	29-Jul-08	<0.1
Kananaskis Lake Upper	21-Aug-08	<0.1
Kananaskis Lake Upper	16-Sep-08	<0.1
Kehiwin Lake	20-Jul-08	0.17
Kehiwin Lake	13-Aug-08	0.42
Kehiwin Lake	2-Sep-08	1.08
Lac La Nonne	12-Jun-08	0.73
Lac La Nonne	4-Jul-08	0.87
Lac La Nonne	25-Jul-08	2.48
Lac La Nonne	18-Aug-08	1.32
Lac La Nonne	12-Sep-08	<0.1
Laurier Lake	24-Jun-08	0.36
Laurier Lake	17-Jul-08	0.18
Laurier Lake	8-Aug-08	0.25
Laurier Lake	26-Aug-08	0.26
Laurier Lake	15-Sep-08	0.13
Long Lake (by Boyle)	9-Jun-08	<0.1
Long Lake (by Boyle)	8-Jul-08	<0.1
Long Lake (by Boyle)	30-Jul-08	0.19
Long Lake (by Boyle)	19-Aug-08	0.21
Long Lake (by Boyle)	9-Sep-08	0.13
Marie Lake	11-Jul-08	<0.1
Marie Lake	9-Aug-08	<0.1
Marie Lake	27-Aug-08	0.1
Marie Lake	22-Sep-08	0.1
McLeod Lake (East)	29-Jun-08	<0.1
McLeod Lake (East)	13-Jul-08	<0.1
McLeod Lake (East)	27-Jul-08	<0.1
McLeod Lake (East)	18-Aug-08	0.12
McLeod Lake (East)	31-Aug-08	0.12
Minnie Lake	20-Jun-08	0.18
Minnie Lake	16-Jul-08	<0.1
Minnie Lake	8-Aug-08	0.11
Minnie Lake	1-Sep-08	0.13
Minnie Lake	16-Sep-08	<0.1
Miquelon Lake	17-Jun-08	0.59
Miquelon Lake	17-Jul-08	0.69

Table A10 Raw Data 2008

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Miquelon Lake	7-Aug-08	0.36
Miquelon Lake	5-Sep-08	0.5
Miquelon Lake	18-Sep-08	0.86
Moonshine Lake Reservoir	3-Jul-08	0.2
Moonshine Lake Reservoir	17-Jul-08	1.56
Moonshine Lake Reservoir	7-Aug-08	0.1
Moonshine Lake Reservoir	25-Aug-08	0.89
Moonshine Lake Reservoir	22-Sep-08	<0.1
Moore (Crane) Lake	3-Jul-08	0.1
Moore (Crane) Lake	25-Jul-08	<0.1
Moore (Crane) Lake	9-Aug-08	0.11
Moore (Crane) Lake	6-Sep-08	<0.1
Nakamun Lake	26-May-08	0.16
Nakamun Lake	24-Jun-08	<0.1
Nakamun Lake	14-Jul-08	0.51
Nakamun Lake	7-Aug-08	0.15
Nakamun Lake	26-Aug-08	2.46
Nakamun Lake	16-Sep-08	0.24
Nakamun Lake	22-Oct-08	0.12
Newell Lake Reservoir	27-Jul-08	<0.1
Newell Lake Reservoir	14-Aug-08	<0.1
Newell Lake Reservoir	28-Aug-08	0.12
Newell Lake Reservoir	24-Sep-08	<0.1
Oster Lake	24-Jun-08	6.46
Oster Lake	21-Jul-08	8.31
Oster Lake	12-Aug-08	14.95
Oster Lake	10-Sep-08	<0.1
Oster Lake	9-Oct-08	0.13
Pigeon Lake	21-Jul-08	<0.1
Pigeon Lake	27-Aug-08	1.15
Pigeon Lake	17-Sep-08	<0.1
Pine Lake	23-Jun-08	0.17
Pine Lake	22-Jul-08	0.3

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Pine Lake	22-Aug-08	0.43
Pine Lake	10-Sep-08	0.81
Reesor Lake Reservoir	23-Jun-08	<0.1
Reesor Lake Reservoir	14-Jul-08	<0.1
Sandy Lake	30-Jul-08	12.92
Sandy Lake	27-Aug-08	13.58
Sandy Lake	25-Sep-08	4.71
Lac Sante	12-Jul-08	0.25
Lac Sante	1-Aug-08	0.11
Lac Sante	23-Aug-08	0.17
Lac Sante	12-Sep-08	0.13
Saskatoon Lake	26-Jun-08	0.33
Saskatoon Lake	27-Jul-08	1.74
Saskatoon Lake	31-Aug-08	5.72
Saskatoon Lake	14-Sep-08	0.37
Siler (Stoney) Lake	3-Jun-08	0.11
Siler (Stoney) Lake	26-Jun-08	0.19
Siler (Stoney) Lake	21-Jul-08	0.18
Siler (Stoney) Lake	22-Aug-08	0.57
Siler (Stoney) Lake	17-Sep-08	0.1
Skeleton Lake South Basin	8-Jun-08	0.11
Skeleton Lake South Basin	6-Jul-08	0.11
Skeleton Lake South Basin	27-Jul-08	0.15
Skeleton Lake South Basin	22-Aug-08	0.75
Skeleton Lake South Basin	27-Sep-08	<0.1
Spruce Coulee Reservoir	23-Jun-08	0.38
Spruce Coulee Reservoir	14-Jul-08	<0.1
Steele (Cross) Lake	25-Jun-08	0.19
Steele (Cross) Lake	23-Jul-08	<0.1
Steele (Cross) Lake	14-Aug-08	0.38
Steele (Cross) Lake	4-Sep-08	1.05
Steele (Cross) Lake	25-Sep-08	<0.1
Sturgeon Lake	26-Jun-08	<0.1

Table A10 Raw Data 2008

Site Name	Date Sampled	MCYST (µg MCLReq./L)
Sturgeon Lake	17-Jul-08	0.18
Sturgeon Lake	7-Aug-08	<0.1
Sturgeon Lake	27-Aug-08	0.55
Sturgeon Lake	10-Sep-08	1.03
Sylvan Lake	8-Jul-08	<0.1
Sylvan Lake	11-Aug-08	<0.1
Sylvan Lake	15-Sep-08	<0.1
Twin Valley Reservoir	29-Jul-08	0.66
Twin Valley Reservoir	19-Aug-08	1.11
Twin Valley Reservoir	30-Sep-08	<0.1
Tyrrell Lake	6-Aug-08	1.19
Wapasu Lake	20-Jun-08	0.46
Wapasu Lake	17-Jul-08	1.71
Wapasu Lake	16-Aug-08	2.96
Wapasu Lake	13-Sep-08	0.34
Wapasu Lake	25-Sep-08	0.82
Winagami Lake	3-Jul-08	<0.1
Winagami Lake	6-Aug-08	0.37
Wizard Lake	27-May-08	<0.1
Wizard Lake	16-Jun-08	<0.1
Wizard Lake	24-Jul-08	0.21
Wizard Lake	13-Aug-08	0.13
Wizard Lake	2-Sep-08	0.13